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The Disturbance of Cell Division Produced by X-rays
at Various Stage of Development of the Frog

By

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One of the first morphological signs of the effect of ionizing radiation on differentiated animal tissues is the suppression of mitotic activity in them. Through the works of Alberti and Politzer (1923), G. S. Strelin (1934), I. V. Bychkovskaya (1956) and others it has been established that in different amphibians X-rays suppress mitoses over a broad dose range (from 2 r). The duration of suppression of mitotic activity is determined first of all by the magnitude of the dose used. Here the age of the organism and the physiological condition of the tissue are also of importance.

It has also been made clear that X-rays within broad dose limits do not stop mitosis at definite phases of it, as occurs with temperature influences and from the effect of certain chemical substances, like, for example, colchicine (Corliss, 1953), lithium (Korovina and Dampel', 1945) and others. The mitoses which have begun before irradiation with X-rays continue after it has been stopped, and the dividing cells go into a resting state. After irradiation the prophases disappear first; then, the metaphase anaphases and, last, the telophases disappear. Therefore, the radiation suppresses the initiation of cell division.

In connection with the fact that in the corneas of adult frogs X-rays suppressed the mitoses even in a dose of 2 r (Bychkovskaya, 1956) data obtained by Ya. L. Shekhtman (1934) and V. A. Blinov (1950) with respect to the effect of large doses on division in amphibians are of particular interest. According to the data of these authors, doses up to 100,000 r do not stop division in amphibians. This fact has made it possible for Blinov to speak of the insensitivity of mitosis to irradiation during the period of division. According to our observations (Kheysina, 1956), doses of 10,000 and 25,000 r do not stop division either, although they slow it and distort it somewhat; however, the embryo continues to develop until the late blastula stage. It should be pointed out that after irradiation of fertilized egg cells of amphibians with doses of 10,000 r and higher we could not find any normal mitosis or chromosomes, although achromatic spindles could be seen. Therefore, with high doses division is carried out by means of "achromosomal" mitosis, that is by a very unusual method. With a dose of 1,000 r no ~~apparent~~ apparent disturbances are observed in the course of mitosis, and the division proceeded in absolute synchrony with division in control non-irradiated ova. Therefore, while in differentiated tissues a dose of 2 r suppressed mitosis, during division in amphibians a considerably larger dose does not have any noticeable influence on it.

Beginning with the works of O. Hertwig (1911), a considerable number of observations ^{has} ~~has~~ been accumulated which show that the embryo at the

early stages of development and the fertilized ovum before the onset of division are most sensitive to the effect of radiation on the ~~body~~ organism (Astaurov, 1947; Rugh, 1954). By comparing the sensitivity of the early stages of development and "the insensitivity" of mitoses at these same stages to the effect of X-rays a contradiction arises, which speaks for period, characteristics of the ~~stage~~ of division/ V. A. Blinov (1954) attempts to explain the insensitivity of division to irradiation by the specific nature of the metabolism at this stage of development.

If in differentiated tissues a reactive suppression of the initiation of cell division is observed, and if this does not exist during division, the question arises as to how the reaction to irradiation is changed during the course of ontogeny with the formation and differentiation of tissues. For the purpose of explaining this question several experiments were performed

During the winter-spring period spawn was ~~was~~ obtained in the laboratory from hypophysectomized frogs, from which embryos of the desired age were cultivated. For the purpose of irradiation the following stages of development of the frog were utilized: first group -- month-old tadpoles after hatching; second group -- five-to-seven-day old tadpoles; third -- tadpoles on the day of hatching (within their membranes and also free of them); fourth -- the neurulas. Two series of experiments were performed with the neurulas; in one, the irradiation dose was 250 r; in the other, 1,000 r. Five series of experiments with a dose of 1,000 r were performed with tadpoles of

the first to third groups. The experiments were performed at room temperature (20-22° C). The irradiation ~~on~~ conditions were the following: voltage 175 kv, current 15 ma, distance from anode to object, 24 centimeters; no filter, dose rate 447 r/minute (RUM-1 apparatus).

The irradiated and control material was kept in open crystallizers with sedimented water. Part of the embryos were fixed with Zenker-formol after irradiation every hour for the first five hours, and then after 24-48 hours and later. Observations were made of the experimental and control embryos, and the times that the embryos died were noted. From the fixed material preparations were made. In addition to sections, total preparations were made from the corneas and from the skin of the tail of five-to-seven-day old and older tadpoles. The total preparations were stained with hematoxylin according to the Yasvoin method, according to the Karachi method and with alum-carmin. The sections (of seven micron thickness) were stained with hematoxylin, and part of them were treated according to the Feulgen method.

In the corneas a count was made of the mitoses for the entire surface. In the skin of the tail the mitoses were counted for each period in 100 microscopic fields (ocular 7x; objective, oil immersion, 90x). The mitoses were counted in 50 serial sections, whereby their numbers were recorded separately in four principal tissues: ectodermal derivatives,

the neural tube, mesoderm and entoderm. The control preparations and sections were treated in the same way as the experimental sections and at the same time.

It was found by observation that the neurulas immediately after irradiation were no different from the controls, and during the first ^{two} ~~five~~ days developed normally, but by the fifth to seventh day the irradiated embryos had already become notably different from the controls in their size, and they began to die out. By the 10th day all the experimental tadpoles had died. In the irradiated tadpoles of the second and third groups the suppression of the anlagen of new organs was observed, and the existing organs continued to develop as in the controls. The irradiated animals were smaller than the controls. Tadpoles of the third group ^{all} died two weeks after irradiation. Tadpoles of the second group began to die out at the end of the first month, and they all died at the beginning of the second month. Tadpoles of the first group proved to be more resistant to the effect of irradiation. The experimental animals ~~went through~~ underwent metamorphosis and continued to live for a long time. They were different from the control tadpoles only in a slight reduction in the body size and their ^{lesser} ~~weaker~~ degree of pigmentation.

In the study of total preparations prepared from the corneas of tadpoles of the first and second groups we found, in all cases beginning with the first hour after irradiation, a reduction in the number of ~~mita~~ mitoses by comparison with the controls. Here, a perfectly regular

reduction occurred in all cases in the total number of mitoses. Chiefly there was a reduction in the number of prophase and metaphases (Fig. 1).

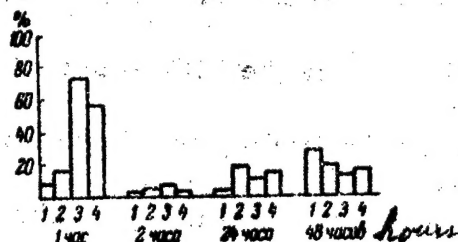


Fig. 1. Change in the Number of Mitoses in the Corneal Epithelium of Tadpoles on the Fifth Day After Hatching at Different Intervals After Irradiation (Dose 1,000 r)

Along the ordinate axis -- the number of mitoses as expressed in percentages of the controls. 1 -- prophase, 2 -- metaphase, 3 -- anaphase, 4 -- telophase.

One or two hours after irradiation the mitoses are encountered chiefly at the late stages -- ana- and telophases. Four to five hours ~~later~~ after a dose of 1,000 r the mitoses disappear completely in the corneas of the tadpoles of all the ages studied. After 24 hours there is a recurrence of mitoses in the irradiated corneas, and at this time all the phases of the mitotic cycle are seen simultaneously. Evidently,

begin again
the mitoses/~~before~~ before the lapse of 24 hours. After 48 hours, seven,
11 and 28 days the mitoses continue to be observed in the irradiated
corneas, but the mitotic activity does not reach the control level.

In the cells of the skin of the tail the mitoses are suppressed after
irradiation, just as in the cornea. The data obtained concerning the
suppression of mitotic activity in the corneal epithelium and skin of
tadpoles in general coincide with similar results obtained by G. S.
Strelin (1934) on older tadpoles.

In the sections prepared from tadpoles irradiated on the day of
hatching (group three) it was found that an hour after the irradiation
there was a notable reduction in the total number of mitoses. After
three hours the mitoses disappear completely in the entoderm and
almost completely in the ectodermal and mesodermal derivatives.
At the same time, the mitotic activity in the neural tube is maintained
at a high level. Four to five hours after irradiation the mitoses are
completely absent in the ectodermal, entodermal and mesodermal
tissues, but they are very distinct and are maintained in considerable
numbers in the neural tube (Fig. 2). The normal interrelationship in
the number of mitoses according to their separate phases does not
allow us to suppose that mitoses in the neural tube are successfully
completed and disappear after irradiation. After 24 hours the mitoses
are again observed in all tissues. Such data were obtained in two para

series of experiments. The count of the total number of mitoses which occurred in the ecto-, ento- and mesodermal derivatives shows, however, that the mitotic activity remains reduced by comparison with the control

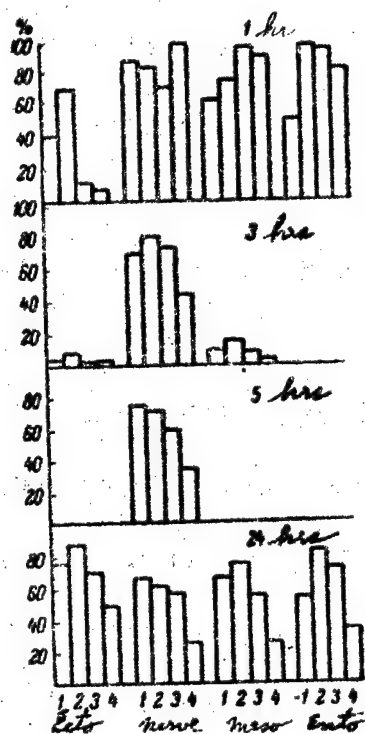


Fig. 2. Reduction and Recovery in Mitotic Activity and Derivatives of Various Embryonic Layers in Tadpoles on the Day of Hatching and at Various Intervals after Irradiation (Dose 1,000). Along the ordinate axis -- the number of mitoses expressed in percentage of the control. The designation of the phases is the same as for Fig. 1. Ecto, ento, meso, nerve -- derivatives, respectively, of ectoderm, entoderm, mesoderm and the neural plate.

In preparations made from the irradiated neurulas (group four), after one to three hours and later mitoses were found in all the embryonic layers. Here, no noticeable reduction in mitotic activity was found either after the use of a dose of 250 r or after the dose of 1,000 r (Fig. 3). The cells of all the embryonic layers of the irradiated neurulas continue to multiply, and the embryos developed in the same way as in the controls.

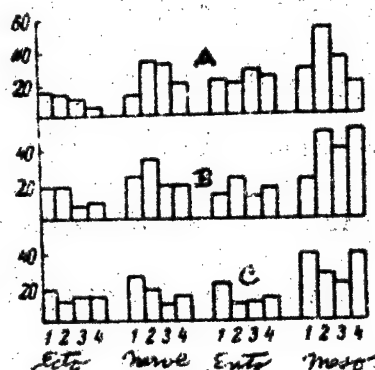


Fig. 3. Number of Mitotic Figures in Absolute Quantities in Various Neurula Layers.

A -- in the control; B -- five hours after irradiation with X-rays in dose of 250 r; C -- five hours after the use of a dose of 1,000 r. The other designations are the same as for Fig. 1 and 2.

The data obtained show that the relative "insensitivity" of mitosis to irradiation, which was established previously for the stage of segmentation in amphibians, is characteristic not only of this stage, but is found also at later stages of embryonic development. The mitotic activity of cells in the neurula stage is not suppressed either by X-rays in a dose 250

and 1,000 r, just as in the stage of segmentation. We cannot ~~stax~~ state that the sensitivity of cell division at this stage of development was not increased by comparison with the segmentation period, because we did not try out the effects of such large doses as were used in the investigation of mitoses during the segmentation period. However, undoubtedly, the sensitivity of cell division at the neurula stage is much less than the sensitivity of mitoses in the tissues of just hatched tadpoles and adult animals. This, probably, can be explained by the fact that at the neurula stage there is no tissue differentiation, the cells of embryonic layers do not possess the capacity of reactive suppression of mitoses. The data presented do not permit us to say that sensitivity of mitosis to irradiation during segmentation of amphibians is qualitatively different (Blinov, 1950) from the sensitivity of mitotic division of cells at other stages of development. The sensitivity of mitosis to irradiation is changed during the course of ontogeny with the differentiation of embryonic layers. The turning point with respect to the sensitivity of mitosis during the development of amphibians is probably the onset of differentiation of the embryonic tissues and the transition of it to ~~active~~ active existence. Therefore, in the tadpole at the time of hatching the reactive suppression of mitosis becomes clearly expressed, and at the neurula stage it has not yet occurred. With a dose of 1,000 r at this later stage of development cell division is not suppressed only in the neural structures -- the brain and spinal

cord. In the derivatives of other embryonic layers a marked inhibition occurs of the initiation of cell division. By the third to fourth hour after irradiation the mitoses in these tissues ^{have disappeared} ~~disappear~~ completely and return after approximately 24 hours. The insensitivity of the mitotic process in nerve tissue of frog ~~embryos~~ tadpoles at the stage where they hatch out is associated with a relatively high degree of sensitivity of nervous system organs which is manifested in their destruction as early as right after irradiation. The particular sensitivity of the neural layer by comparison with the others has already been noted (Zavarzin, Yasvoin, Aleksandrov, Strelin, 1937); we have shown it also. During segmentation in amphibians mitosis is insensitive to irradiation, and the survival rate ~~of~~ of irradiated embryos at this stage is particularly low. According to our data, ~~irradiation of the~~ irradiated neurulas died at shorter intervals ^{older} than embryos, although X-rays did not suppress mitoses in various embryonic tissues.

If we imagine that these relationships are, in general, characteristic of undifferentiated, embryonic layers, selective resistance of the neural layer (in early tadpoles) with respect to the suppression of mitotic activity ~~of irradiation~~ by radiation finds its explanation in the fact that this layer remains poorly differentiated longer than ~~than~~ the others.

It is of great interest to clarify whether rules and regulations similar to those which we have described in amphibians occur during the course of ontogeny in the higher vertebrates.

Conclusions

1. After X-irradiation in a dose of 1,000 r the time needed for extinction of the embryo is determined by its age; the younger the stage of development of embryo the quicker it dies.
2. In neurulas following irradiation the mitotic activity is maintained in all embryonic layers.
3. In just hatched tadpoles the mitotic activity is suppressed three to four hours after irradiation in all tissues, with the exception of the neural tube.
4. In the corneal epithelium and skin of the tail of five to seven and 30-day-old tadpoles mitotic activity is suppressed completely three hours after irradiation.
5. Preservation of mitotic activity in the neural tube of the just hatched tadpole and in all the tissues of the neurula can be explained by the absence of a specific differentiation in these tissues.

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Cytochemistry of Sensory and Motor Cells of the Spinal
Cord System of the Chick Embryo in Connection with
Characteristics of their Function at Various
Stages of Development

By

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Introduction

Functional histochemical and cytochemical investigations require, of necessity, the comprehensive study of the cell which includes the following: morphological investigations, study of the metabolism and function.

At the present time, in the world of microscopic and ultramicroscopic magnitudes morphology, physiology and biochemistry are so closely associated that the need for a comprehensive study of the cell is being

recognized to a progressively greater extent. Cytology is gradually becoming a synthetic science.

For histochemistry an indisruptible association between biochemistry and morphology is characteristic, because the problem of cytochemistry is not simply the study of the chemical composition of organs, tissues, and cells but rather the study of the localization and concentration of various chemical agents and their qualitative and quantitative changes during the course of metabolism in certain structures: the cells of the organoids, inclusions, interstitial substance. Therefore, the histochemical investigation is, to a great degree, a morphological investigation.

In order to explain rationally the characteristics of the organization and chemical composition of microscopic structural elements of the organism

it is necessary to establish their functional specifications. Therefore, the main task of functional cytochemistry is a study of the rules and regulations associating the organization, metabolism and specific function of the microscopic structures of the organism (Levinson and Leykina, 1956).

A. L. Shabadash (1958) also believes that the ultimate task of the histochemical investigation is the detection of comprehensive signs of the functional states of the cells and tissues.

Histochemical investigation of the nervous system is particularly important, because even ⁱⁿ the smallest quantities of it which can be subjected to biochemical investigation there are many genetically, morphologically, and chemically distinct neurons, neuroglia, blood and lymphatic vessels, etc.).

Nurnberger and Gordon (1957) isolated cell nuclei from different

brain centers of white rats by means of fractional centrifugation and showed that the number of neuron nuclei amounts to about 85% of the total number of nuclei. In the cerebral cortex of the white rat, of the Macacus rhesus monkey and man, where the density of nerve cells is practically the highest for the brain, the neuron nuclei amount to only 14-26% ^{of the} total number of nuclei. G. I. Roskin and others (1953-1954) showed that functionally different neurons are distinguished in their chemical composition. These data once again show the importance and necessity of morphological analysis for the purpose of demonstrating the finer connections between the chemical composition and function.

The development of functional biochemistry of nerve tissue in laboratories of the Academician A. V. Palladin (1952, 1954, 1956, 1957), G. Y. Vladimirov (1954) and Ye. M. Kreps (1952, 1955, 1956) also led to the ^{idea of the} need for a comprehensive study of the problem.

The principal works in the field of functional histochemistry and cyto-

chemistry of nerve tissue were performed by A. L. Shabadash (1949, 1953, 1957, 1958), G. I. Roskin and others (1953, 1954, 1958), Ya A. Vinnikov and L. K. Titova (1957, 1958), V. V. Portugal^o/v (1955), Hyden (1943, 1947, 1955); and a number of works has been published by us and our co-workers (1956, 1957, 1958).

For the purpose of studying the functional histochemistry of ^{nerve} tissue the experimental method is usually used. By one method or another (by means of electric, sound and other stimuli) various portions of the nervous system were put into a state of excitation, and then the content and distribution of various substances were compared, chiefly RNA in the nerve cells which were in a state of relative rest and in a state of excitation or fatigue (Hyden, 1943, 1955; Hamberger and Hyden, 1945, 1949; Bertram and Barr, 1949; Brattgard, 1951; Brattgard and Hyden, 1954; Bogoyavlenskii, 1954; Migunova, 1955, 1956; Vinnikov and Titova, 1957, 1958; Brodskiy and Nechayeva, 1958a).

The comparative method was used by G. I. Roskin and others and A. L. Shabadash^h (1949). They made a study of the concentration of a number of substances in neurons, which were different in their function and positionⁱ in the nervous system, and showed that these neurons were different from one another in their cytochemical characteristics.

In the present work we decided to proceed along a different route. During the course of embryonic development changes occur in the function of nerve cells. Kuo (1932, 1938), Windle and Orr (1934) and, particularly, Volokhov (1951, 1959) distinguished functionally different stages in the embryonic development of a number of vertebrates, particularly birds, which can be characterized by different types of motor reactions: spontaneous passive, spontaneous active and, finally, reflex. Simultaneously, the

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morphogenesis of the nervous system occurs also: efferent processes of the neurons grow out and reach the innervated substrate; at a certain stage of development reflex arcs are formed, etc. The change in the motor activity of the muscles is inevitably expressed in the specific activity of the neurons innervating them. Therefore, study of the cytochemistry of nerve cells at the turning points in the development of the nervous system, when the intensity and the nature of the function of various neurons change, can reveal the specific connections and interrelationships between the form, function and change in the chemical composition of nerve cells.

Material and Methods

We made a study of motor cells of the anterior horns of the spinal cord and the sensory cells of spinal ganglia of chick embryos of the white leghorn strain at different stages of development.

The cervical portion of the spinal cord was fixed with the corresponding spinal ganglia. The embryos in the early stages were fixed as a whole. The ova were developed in an incubator. The following embryos were examined: those of the third, fourth, fifth, sixth, seventh, eighth, ninth, eleventh, thirteenth, fifteenth, seventeenth, nineteenth, twentieth, and twenty-first days of incubation and a one-day-old chick.

The localization and distribution of DNA were studied (Feulgen reaction), RNA (Brachet), histidine (diazotization reaction), arginine (Serr), glycogen (Shabadash reaction), alkaline phosphatase (Gomori reaction), thiol compounds (Chevrement Frederic reaction).

Results

On the fourth day of development spontaneous active movements of the

L

head of the embryo begin (Volchov, 1951). Apparently, they are caused by humoral influences on the motor cells of the anterior horns of the spinal cord, which at this time are formed from a mass of neuroblasts (Ramon y Cajal, 1890). The statement by L. S. Gol'din (1949) that at this time groups of motor cells form a symplast is incorrect; apparently, the author studied sections which were too thick. Cells of special ganglia at this time are not yet connected with the motor ganglia, and the reflex arc has not been formed as yet.

At this time, changes are observed in the content of a number of chemical substances in the motor cells. On the third day the concentration of RNA in the bipolar neuroblasts is very great; their cytoplasm stains diffusely and very intensely with p. ronine. On the fourth day the concentration of RNA decreases considerably. Differentiation of nerve cells in the spinal cord of the chick proceeds in a cephalo-caudal direction. On the fourth day, the motor cells are differentiated in the anterior horns only in the cervical thickening. They innervate the muscles of the neck and the anterior extremities. Only in these motor cells is a reduction in the RNA concentration observed. The neuroblasts of the anterior horns of the spinal cord which are situated further in a caudal direction still are not carrying out any special functions, and / no reduction / of the RNA concentration in them occurs (Fig. 1). Apparently, the onset of specific activity of motor cells is associated with the disintegration of RNA, which under these conditions is not compensated by a / corresponding synthesis (Kedrovskiy, 1951).



Fig. 1. Anterior Horn of Spinal Cord of Chick Embryo on Fourth Day of Incubation. 1- In the cervical thickening; 2- In the thoracic portion. Staining with methyl green-pyronine. Photographed in a comparison microscope. Magnification 720x.

Clear-cut results were given by the reaction for alkaline phosphatase. On the third day of incubation a weak reaction was observed only in the nucleoli; on the fourth day, the black precipitate was very pronounced in the form of clumps and was seen also in the cytoplasm. Moog (1943) also found a change in the activity of both alkaline and acid phosphatases in the motor cells on the fourth day of incubation. Her data have been confirmed by Barron and Mottet, (1951).

At the same time, the intensity of the reaction for thiol compounds, which is expressed well in the cell nuclei, is somewhat intensified.

In these spinal ganglia, on the fourth day of incubation, there are no differentiated ganglion cells; there are only neuroblasts. No changes occur in the content and distribution of chemical substances in the cells of the spinal ganglia, because the latter are still not connected with the motor neurons and do not carry out any specific functions.

The problem of the nature of spontaneous active movements of the chick embryo has given rise to disputes. Some authors, (Windle and Orr, (1934) believe that spontaneous active movements are of a myogenic nature; others (Visintin and Levi-Montalcini, 1939; Volokhov, 1951; Chumak, 1958) believe that they are neurogenic, arising in response to internal chemical agents on motor nerve cells rather than on muscle. Our data show that in connection with the onset of spontaneous active movements of the neck and anterior extremities in the chick embryo on the fourth day of incubation there is a change in the cytochemical characteristics of the motor nerve cells, which innervate the corresponding transversely-striated muscles, whereas in the neuroblasts located in the thoracic portion of the spinal cord and which are not as yet carrying out any specific functions no changes are observed in the RNA content and in the thiol compounds or in the activity of the alkaline phosphatase.

Finally, in the sensory nerve cells in the cervical portion which are still undifferentiated and incapable of specific activity there are no histochemical changes found. Therefore, the onset of spontaneous active movements are associated both with morphological (Ramony Cajal, 1890) and cytochemical changes in the cells which innervate the corresponding skeletal muscles.

The facts and considerations presented permit us to adhere to the idea of A. A. Volokhov (1951) that the nature of the early spontaneous active movements of the chick embryo are neurogenic.

An important turning point in the development of the chick embryo and in the functioning of the nervous system occurs on the seventh day of development. All the authors (Kuo, 1932, 1938; Windle and Orr, 1934;

Volokhov, 1951, 1959 and others) who studied the motor activity of the embryo, point out the fact that on the seventh day of development the first reactions are observed to external stimuli, reflex reactions appear, a reflex arc is formed, and connections are established between the spinal cord and the periphery. The cell processes of the spinal ganglion reach the skin, and tactile exteroceptive pathways are formed (Levi-Montalcini and Levi, 1943).

Up to the seventh day of incubation there is no connective tissue between the nerve cells of the spinal ganglion; after impregnation by the ^eFoots method various fine reticulin fibers are seen in the embryonic connective tissues surrounding the spinal ganglion. On the seventh day of incubation the connective tissue begins to penetrate into the ventrolateral portion of the ganglion. The reticulin fibers form capsules around the marginal cells, and afterwards the fibers pass between the cells. On the eighth day, all the cells of the ventrolateral portion of the ganglion are already surrounded by reticulin fibers and by cells of embryonic connective tissue (Fig. 2). Therefore, on the seventh day a specialization of the cells of the spinal ganglion and a differentiation of them from neuroblasts begins.

On the seventh day of development in connection with the onset of functioning of the reflex arc essential changes are observed in the content and distribution of a number of chemical substances in the nerve cells along with morphological changes of them.

In the cytoplasm of the motor and sensory cells the concentration of RNA (Fig. 3) increases considerably. It should be kept in mind that the intensity of the staining with pyronine becomes greater despite the fact that between the sixth and seventh day the cells increase quite considerably in size.

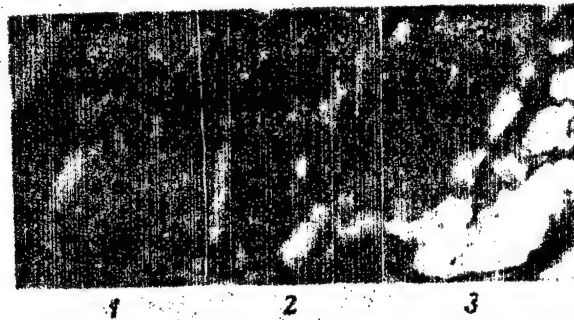


Fig. 2. Spinal Ganglion of Chick Embryo at the Level of the Cervical Thickening
1- On the sixth; 2- On the seventh; 3- On the eighth day of incubation.
Silvering according to the Foote method photographed in a comparison microscope. Magnification 720x.

Between the cells of the spinal ganglion there are differences with respect to the concentration of RNA in the cytoplasm. In the spinal ganglion up to the fifteenth day of development there are two types of cells (Levi-Montalcini and Levi, 1943, Hamburger and Levi-Montalcini, 1944).

The large differentiated cells are located ventrolaterally; the small poorly differentiated cells are located mediodorsal. The increase in the RNA concentration occurs only in the large, differentiated, already morphologically specialized neurons which are already beginning to function specifically. In the small cells, neuroblasts, which are still undifferentiated and not carrying out any specific functions the concentration of RNA is unchanged on the seventh day.

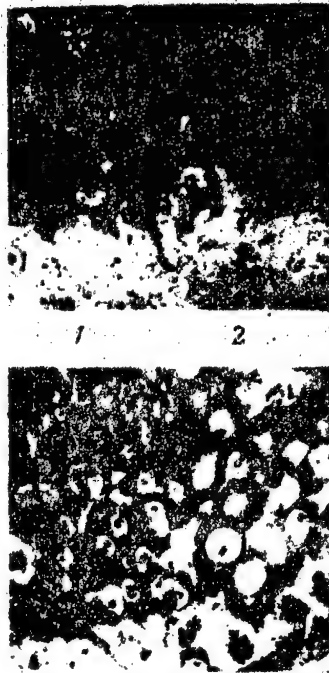


Fig. 3. Motor Cells of the Anterior Horns of the Spinal Cord (a) and Sensory Cells of the Spinal Ganglion (b) of the Chick Embryo

1- On the sixth; 2- On the seventh day of incubation. Staining with methyl green-pyronine. Photographed in a comparison microscope. Magnification 720x.

An important part in the metabolism of nerve cells is played by glycogen (Shabadash, 1949). Up to the seventh day of development, even with such a sensitive method as the Shabadash reaction it was impossible to demonstrate glycogen either in the motor or sensory cells. On the seventh day, glycogen is demonstrated well in the cytoplasm of both types of cells of the spinal ganglion, whereby ⁱⁿ differentiated cells there is more of it than in the poorly differentiated cells (Fig. 4).



Fig. 4. Motor Cells of the Anterior Horns of the Spical Cord (a) and Sensory Cells of the Spinal Ganglion (b) of the Chick Embryo
1- On the sixth; 2- On the seventh day of incubation. Reaction for glycogen.
Objective 90x; ocular 10x.

The concentration of histidine in the cytoplasm of motor and sensory cells increases considerably on the seventh day. In the nuclei a redistribution of it occurs; on the sixth day the ^{amino}acid is distributed throughout the nucleus and against a diffuse background small clumps ^{of} chromatin and nucleoli containing histidine in a somewhat greater concentration are distinguished poorly. On the seventh day, the histidine is practically not found in the ^{cyop} karyoplasm of the majority of nuclei of motor and sensory cells; on the other hand, its concentration is increased in the chromatin and nucleoli (Fig. 5).

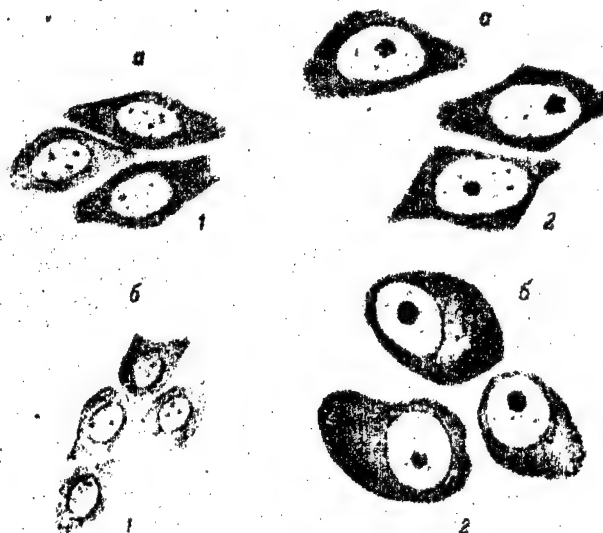


Fig. 5. Motor cells of the anterior horns of the spinal cord (a) and sensory cells of the spinal ganglion (b) of the chick embryo

1) on the sixth day;
 2) on the seventh day of incubation.
 Test for histidine.
 Objective: 90x; ocular, 10x.

On the seventh day of development, the activity of the alkaline phosphatase is notably altered in neurons of the spinal ganglion.

On the sixth day, a weak reaction occurs only in the nucleolus in the motor and sensory cells. The cytoplasm remains perfectly clear.

On the seventh day, a large quantity of dark granules is seen in the cytoplasm of large differentiated sensory cells, in the ventrolateral portion of the ganglion, whereas the small cells give practically no reaction.

Moog (1943, 1944) also established the presence of a difference in the intensity of reaction for acid and alkaline phosphatases between small and large cells in the spinal ganglion of the chick embryo. She believes that chemical differentiation of nerve cells proceeds in parallel with hist

logical differentiation, which is also confirmed by our data.

In the study of the localization of the thiol compounds in the motor neurons on the sixth and seventh day of development we came across an interesting type of histochemical differentiation of cells. On the sixth day, the thiol compounds are ^{located} chiefly in the nucleus, whereby after performing the test it stains diffusely; in the cytoplasm the stain is much weaker. On the seventh day the distribution of thiol compounds in the nucleus is completely different: they are concentrated in the nucleolus and, apparently, in a few clumps of chromatin; the entire karyoplasm is without any reaction at all and remains clear. In the cytoplasm the intensity of the reaction increases considerably (Fig. 6). In this case we can not speak of changes in the concentration of substance in the cell but only of ^{its} redistribution among cellular structures during the course of functional development of the neurons.



Fig. 6. Motor Cells of Anterior Horns of the Spinal Cord of the Chick Embryo. 1- on the sixth; 2- on the seventh day of incubation. Tests for thiol groups. Objective 90x; ocular 10x.

Therefore, on the seventh day of development the chick embryo, in connection with the formation of a reflex arc and the beginning of reflex movements, there is a change in the character and intensity of the specific activity of the motor cells of the spinal cord, and a specific activity of

7
the afferent neurons of the spinal ganglion appears. Thereby, chemical differentiation is observed in both types of neurons: there ^{is} an increase in the concentration of RNA in the cytoplasm and in the nucleolus, the concentration^s of histidine, glycogen, alkaline phosphatase^a are increased, and a redistribution of the thiol compounds is observed.

With the further development of the embryo on the eight, ninth, and tenth days of incubation a certain lessening of the concentration of the substances studied was observed in the motor and sensory cells.

On the eleventh day, changes occur again in the content of a number of substances^u: the concentration of RNA is increased (somewhat more in the sensory cells and less in the motor cells) (Fig. 7), in both types of cells there is a marked increase in the intensity of the reaction to histidine and arginine.

After the seventh day the test for glycogen becomes negative. On the eleventh day the glycogen is demonstrated in the form of quite numerous clumps in the large more differentiated neurons of the spinal ganglion lying in its ventrolateral portion, whereas in the small, poorly differentiated cells it is absent. In the motor cells this reaction is very weak.

The test for alkaline phosphatase^a is very weakly expressed on the eleventh day in the motor cells, whereas the spinal ganglion is distinguished by more intense reactions throughout the entire section through the embryo. With respect to the activity of this enzyme two types of cells are readily distinguished: in the large cells the activity is considerably greater than in the small cells.

through the fourteenth day a reduction in the embryonic activity occurs, and from the fifteenth through the eighteenth day a rest period occurs.

Cytochemical observations for the most part confirm this division into periods. The concentration of RNA in the motor and sensory cells at this time gradually decreases (Fig. 7), which may be associated both with a reduction in the metabolism as a result of ^a reduction in the specific activity and with the continuing differentiation of the neurons.

It should be noted that at this time there is a gradual reduction in the difference in concentration of RNA between the large and small cells of the spinal ganglion and subsequently they do not differ from one another in their cytochemical indices. This is probably explained by the fact that beginning with the fifteenth day the mediodorsal cells mature, and in their degree of differentiation do not differ from the ventrolateral cells (Levi-Montalcini and Levi, 1943).

The quantity of glycogen also decreases after the eleventh day until there is a negative reaction in the cells of the spinal ganglion and a hardly noticeable one in the motor cells. Only on the seventeenth day do small clumps of glycogen reappear in the individual motor, sensory cells. The intensity ^{of} the reaction for histidine, thiol compounds and alkaline phosphatase ^a also decreases notably.

The most important phase in the development of the motor activity—and intensification of it—occurs on the nineteenth-twentieth day. ^{By} this time the morphological formation of the fetus is completed for the most part, and the rest period terminates and a period of pre-hatching begins; then (twentieth to twenty-first day) hatching occurs (Kuo, 1938). In connection with this, both the specific activity of the motor and sensory cells



Fig. 8. Motor cells of the anterior spinal crescents (a) and the sensitive cells of the spinal ganglion (b) of a chick embryo.

- 1) on the 17th day;
- 2) on the 19th day of incubation;
- 3) in a one-day chick.

Stained with methyl green-pyronine. Taken in a comparison magnified 720 times.

are increased, and the changes in their chemical composition increase.

The concentration of RNA in the cytoplasm and nucleus of both types of cells increases considerably (Fig. 8 a notable quantity of glycogen appears (Fig. 9), the intensity of the action for histidine, thiol compounds (Fig. 10 increases, and the reactivity of the alkaline phosphatase increases.

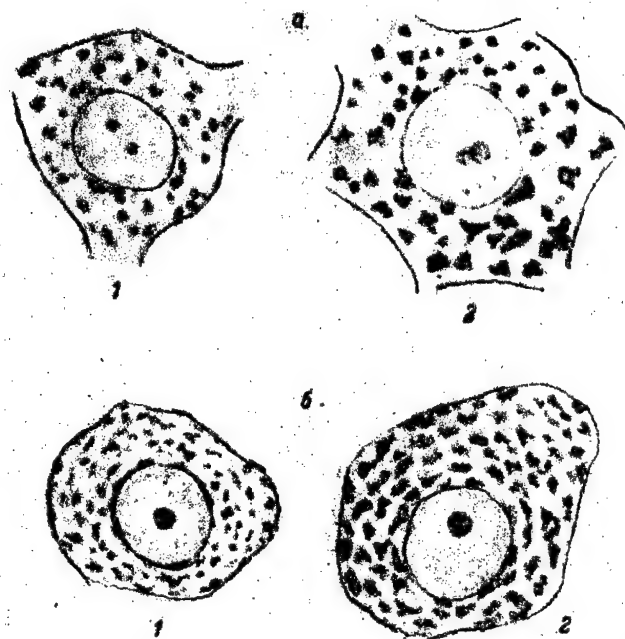


Fig. 9. Motor cells of the anterior horns of the spinal cord (a) and sensory cells of the spinal ganglion (b) of the chick embryo.

1) on the 20th; 2) on the 21st day of development. Test for glycogen. Objective 90x; ocular 10x.

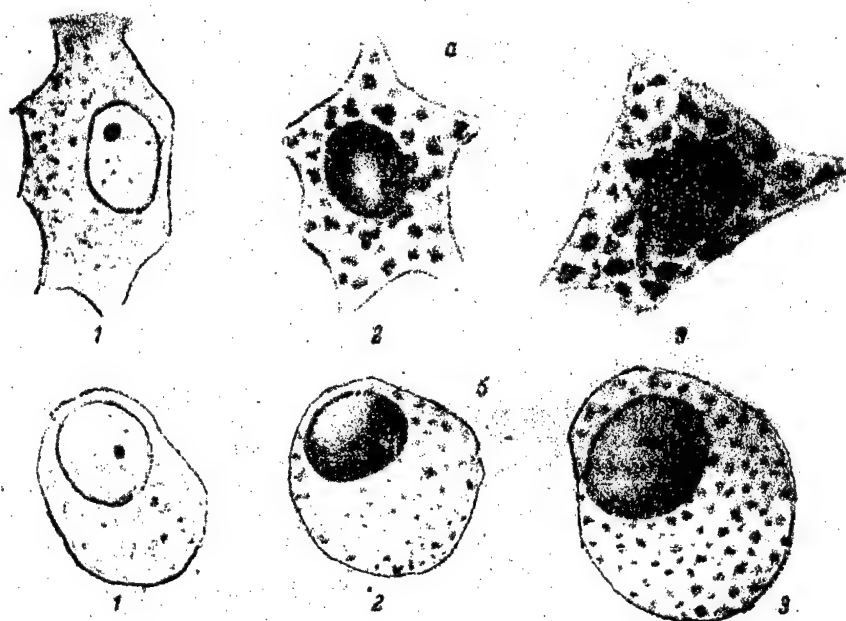


Fig. 10. Motor cells of the anterior horns of the spinal cord (a) and sensory cells of the spinal ganglion (b) of the chick embryo.

1) on the 19th; 2) on the 20th; 3) on the 21st day of incubation. Test for thiol groups. Objective 90x; ocular 10x.

A further intensification of the functions ^{occurs} on the twentieth-twenty-first day. This is the period of hatching, when the fetus makes very strong movements. With pecking and breaking of the shell a change occurs in the position of the head and the upper cervical vertebrae of the chick (Lippincott, 1932), that is, the muscles work which obtain their innervation from the section of spinal cord which we are studying. In connection with this a further histochemical re^{organization} of the corresponding neurons is also observed. ^{In the} pecking (twenty-first day) and one-day-old chick the concentration of RNA in the motor and sensory cells is increased (Fig. 8). There is also a considerable increase in the quantity of glycogen. While on the twentieth day the glycogen does not exist in all the cells of the spinal ganglion on the twenty-first day clumps of glycogen are found in all the sensory neurons. In the one-day-old chick a further increase in the quantity of glycogen is observed in the cytoplasm of the neurons (Fig. 9).

As has been shown in our laboratory by V. N. R. Larin, reactions for histidine α increase somewhat on the twentieth day in the motor cells and on the twenty-first day also in the sensory cells. The reaction for thiol compounds increases somewhat only in the motor cells on the twenty-first day; afterwards, its changes are slight (Fig. 10). The activity of alkaline phosphatase ^a is generally unchanged.

As is seen from what has been presented above, the concentration of RNA, glycogen, histidine, thiol compounds and alkaline phosphatase activity in the neurons changes at various ^{stages} of development in connection with the change in the motor activity of the embryo.

The same rules and regulations can not be found with respect to DNA and

arginine. The reaction^s for arginine on the sixth, seventh, eighth day of development are of moderate intensity. Afterwards, this reaction gradually increases. If we compare preparations of two successive stages, the differenceⁿ proves to be insignificant; if we compare the intensity of the reaction in the cytoplasm and in the nucleus of motor and sensory cells on the sixth and twenty-first day of development it is clearly seen that the concentration of arginine at late stages is considerably higher in the cytoplasm, nucleolus and nuclear granules and much less in the karyoplasm.

M. Ye. Struve (1953, 1955) studied arginine in the motor cells of the spinal cord at various stages of fetal development of the rabbit and also established the fact that with the development of the embryo a gradual increase is observed in the intensity of the reaction in the cytoplasm, protein granules of the nucleus and nucleoli. The change in the quantity of arginine and histidine was established by biochemical methods at certain stages of development of amphibians (Dorfman, 1958).

The DNA content apparently changes in the nuclei very gradually also. No marked changes can be found. On the seventh day of development

DNA is arranged in the form of granules in the nucleoli and near the nuclear membrane; in the k_aryoplasm individual small granules are seen; this applies to the nuclei of motor cells. In the spinal ganglion two types of cells are readily distinguishable with respect to their content of DNA; in the nuclei of the large differentiated cells of the ventrolateral portion of the ganglion only several granules of DNA are seen which are located chiefly near the nucleolus. The nuclei of poorly differentiated small cells of the mediodorsal portion of the ganglion contain considerably more Feulgen-positive granules. Along with the round granules

many small granules are seen which are scattered throughout the entire nucleus. Subsequently, a reorganization of the nuclei occurs in connection with differentiation of the neurons, and if we compare the nuclei of both motor and sensory cells on the seventh and seventeenth day of development it may be noted that the ~~mm~~ number of granules is reduced by the seventeenth day.

Baffoni (1954), studying the DNA in the Purkinje cell nuclei of the cat, concluded that during the course of development of the fetus the nuclei become poor in DNA. A. A. Zhirnova (1955) believes that the DNA content is reduced in the nuclei of the neurons of the anterior horns of the spinal cord and motor area of the cerebral cortex of the rat during the course of embryonic development of the organism.

The problem in the reduction in the quantity of DNA in the nuclei of nerve cells of the developing organism requires further investigations by means of exact quantitative methods.

Special attention has been directed to the histochemistry of the tigroid. The question of the actual existence of ^{Misel} granules in the live cell ^{has} given rise to doubt until recently (Brodskiy, 1956). Recently the work of Dietch and Murray (1956) has appeared; they studied the ^{tigroid} in living cells of the spinal ganglion of the chick embryo in a tissue culture by means of a phase-contrast microscope. They showed that the ^{Misel} bodies are already present in living neurons and, by and large, are not changed after good ^{cyt} ~~cytological~~ fixation. I. P. Belova in our laboratory has shown the existence of ^{tigroid} /in living cells of a number of invertebrate and vertebrate animals by means of the phase-contrast microscope. A. L. Shabadash (1958), in analyzing this question thoroughly, regards the ^{tigroid} as a specific organoid of the nerve cell. The actuality of the existence of the

^{tigroid}, observed in living nerve cells regularly formed during the course of ontogeny and regularly altered depending on various physiological positions, can not be doubted at the present time, in our opinion.

During the course of ontogeny a differentiation occurs in the neurons and the formation of ^{tigroid} /in them. In recent years, a number of works have appeared devoted to this problem.

Ye I. Kalinina (1952, 1956) has shown that in the motor cells of the anterior horn of the spinal cord of rabbits the ^{tigroid} /is demonstrated for the first time on the seventeenth day of development and accumulates in an increased manner ^{during} / the period of occurrence and development of reflex activity. Baffoni (1954) studied the formation of tigroid in the Purkinje cells of mammals. He showed that in a diffusely basophilic neuroblast a peripheral, ^{basophilic} zone is first formed and then various clumps appear the quantity of which increases, and then they assume a shape characteristic of the ^{tigroid} / of ^{Purkinje} /cells of adult animals; thereby, basophilia of the cytoplasm gradually is reduced ^{among} / the clumps. A. A. Zhirnova (1955) showed that the first clumps of ^{tigroid} /formed in the motor neurons of the spinal cord of the rat embryo are seen only on the sixteenth day. With the growth of the embryo and the development of its motor activity an increase occurs in the quantity and size of the ^{Nissl} / clumps. The shape and arrangement of the ^{tigroid} / bodies are changed also during the differentiation of spinal ganglion cells in tissue culture (Dietch Murray, 1956).

The first data concerning the cytochemistry of the developing tigroid were given by M. Ye Struve (1955). In the motor cells of the rabbit embryo she found histidine in the tigroid clumps on the thirteenth day of develop-

ment, and arginine on the twenty-third day. In the pyramidal cells the existence of histidine was determined on the twenty-third day of development, whereas arginine could not be found.

The chemical composition of the tigroid is represented in the following form at the present time. It includes ribonucleoprotein which contains RNA and basic acidophilic protein which is rich in diamino acids, arginine, histidine and lysine and, in the opinion of Linarenko, proteins which show a neutral or acid reaction and contain aromatic amino acids (Bracat, 1944; Roskin, 1945; Caspersson, 1950; Shabadash, 1953; Linarenko, 1953, 1956, 1957). In addition, A. L. Shabadash (1949) showed that glycogen is included in the composition of the tigroid of a number of nerve cells. Soon afterwards, Schubel (1955) confirmed his data. We also have had an opportunity to find glycogen repeatedly in the tigroid of appropriate neurons. Vraa Jensen (1956) believed that the chemical composition of the tigroid is the same in all neurons. We can not agree with this, at least because glycogen in some cells is included in the neurons; in others it is absent.

On the fifth day of development the formation of ^{the} tigroid is begun in the motor neurons of the spinal cord. While the cytoplasm up to this time is diffusely basophilic, on the fifth day the stain loses its uniformity. The accumulations of RNA frequently are located on one side of the nucleus or have the shape of a rim.

On the seventh day, the inhomogeneity of the staining of the cytoplasm becomes more distinct. This occurs both because the clumps become larger and more distinct and because of the paler staining of the cytoplasm among them. Afterwards, the clumps increase in size, and the concentration of RNA in

them increases; among the Nissl bodies in the cytoplasm the concentration of RNA decreases, but does not entirely disappear as has been stated by Barffoni (1954). The cytoplasm remains weakly basophilic.

The presence of RNA among the tigroid clumps was first established by V. Ye Brodskiy (1956) whose data we are able to confirm. The tigroid substance, typical of motor neurons of the adult animal (characteristic shape and arrangement of the clumps, concentration of RNA in them) is formed only on the fifteenth-seventeenth day of development (Fig. 11).

In the first stages of development the reaction for histidine and arginine in general gives a uniform staining of the entire cytoplasm. Only on the ninth day in the motor and on the fifteenth day in the sensory cells

is histidine found in the tigroid in a greater concentration and then in the rest of the cytoplasm, just as occurs in the motor neurons of the spinal cord of the adult chick. A higher concentration of arginine was shown in the tigroid of the cells studied only on the fifteenth-seventeenth day.

An intense reaction for amino acids may depend on two factors: on the relative concentration of the amino acids detected in the protein and on the protein concentration in this structure. Hyden (1943) did not find any essential difference in the concentration of proteins in the tigroid by comparison with the protoplasm; therefore, the intensity of the reaction speaks for an increased concentration of histidine and arginine in the tigroid protein, which is in agreement with the data in the literature. On the twentieth day of development the reaction for thiol compounds coincides with the tigroid substance.

The clumps of glycogen are distributed in the cytoplasm more or less uniformly up to the seventeenth day. Beginning with the twentieth day, and particularly with the twenty-first day of development, glycogen is bound entirely to the tygroid. After the test for glycogen clumps are seen which coincide in their shape, size and location with the tygroid clumps. It is interesting that as a result of fixation these clumps do not shift to one of the margins of the cells, as is frequently observed in other cells⁵ rich in glycogen, (for example, liver cells). This phenomenon, which was very prominent in the cells which we studied on the eleventh-fifteenth day, depends on the fact that glycogen at a certain stage of development of the neurons is apparently associated with the tygroid proteins.

Therefore, during the course of embryonic development not only the shape and size of the tygroid clumps are changed but also its chemical composition: there is a gradual increase, at first, in the concentration of RNA, and then of histidine and arginine. Both these amino acids, as is well known, are included in large quantities in the basic protein of the tygroid and, therefore, this protein approaches the tygroid protein of the adult organism in its chemistry only toward the seventeenth day of development (within the limits of the possibilities of the methods used). Glycogen, which is so characteristic of the tygroid of motor and sensory cells in the spinal cord of the adult animal, appears in the tygroid of the embryo only on the twentieth-twenty-first day.

The histochemical and apparently functional differentiation of the tygroid is completed (within limits of the components studied) only shortly before the end of the fetal development of the animal.

G. I. Roskin and others (1953, 1954, 1958) have established in a number of works that differences exist between motor and sensory cells /^{in the} relationship, concentration and cytology of a number of substances. A. L. Shabadash (1949) showed that the presence or absence of glycogen characterizes nerve cells of different types.

From a comparison of our material with the data in the literature it is seen that the histochemical differences found for the functionally distinct neurons of adult animals are created as early as during the course of embryonic development.

Before the twentieth day of development the concentration of RNA in the sensory cells is higher than in the motor cells. On the twentieth and twenty-first day of embryonic development and in the one-day-old chick no such difference can be found in the RNA concentration, which is characteristic of the adult animal.

On the seventh-eleventh day of development the content of glycogen in the cells of the spinal ganglion proved to be greater than in the motor cells. Afterwards, no clear-cut differences can be found.

Beginning with the first and up through the twentieth day of development, as has been established in our laboratory by V. N. Larina, the histidin concentration in sensory cells is greater^{than} or the same^{as} in the motor cells. Only on the twenty-first day does the concentration of histidine in the motor cells become greater than in the sensory cells for the first time, as is characteristic of adult animals. G. I. Roskin has determined that the same interrelationship exists in mammals.

On the fourth and sixth days the activity of the alkaline phosphatase in sensory and motor cells is the same, in general, but beginning with the

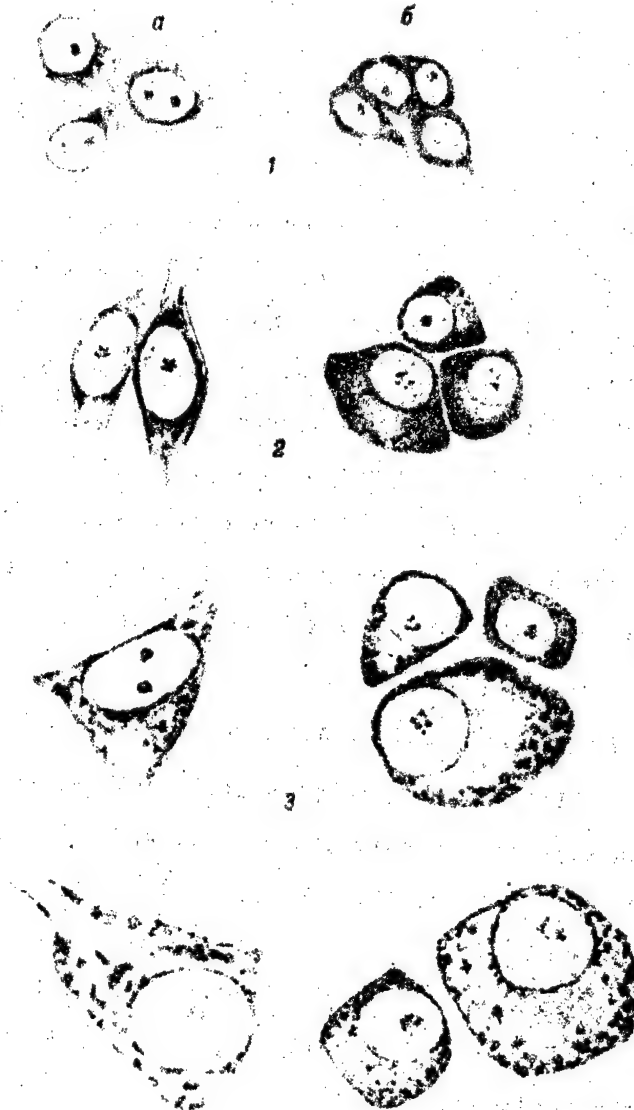


Fig. 11. Formation of the tigroid substance in the motor cells of the anterior horns of the spinal cord (a) and sensory cells of the spinal ganglion (b).

1) on the fifth; 2) on the seventh; 3) on the thirteenth; 4) on the seventeenth day of incubation. Methyl green-pyronine. Objective 90x; ocular 10x.

seventh day of development the activity becomes greater in the sensory cells and this relationship continues during the subsequent development of the embryo and in the one-day-old chick. The same results in adult animals were obtained by G. I. Roskin and M. V. Shornikova (1953).

Up to the fifteenth day of development the concentration of thiol compounds in the sensory and motor cells is approximately the same; from the fifth through the thirteenth day it is somewhat greater in the sensory cells; then the difference becomes smoothed out. According to the data of G. I. Roskin (1954), in mammals the concentration of SH-groups is greater in the motor cells.

Therefore, for certain substances (RNA, histidine) the interrelationship of their concentrations in functionally different neurons characteristic of adult animals is established only at the very end of fetal development; for others (glycogen, thiol compounds), only the eleventh-thirteenth day, that is, at the beginning of the second half of the embryonic period, and, finally, the interrelationship between the activity of alkaline phosphatase in motor and sensory cells is, in general, the same in the embryo and in the adult animal.

Conclusion

During the course of embryonic development, not only growth and differentiation of nerve cells and changes in their size, shape and organization associated with this occur but there is also a change in the chemical composition of neurons, whereby these changes are produced functionally.

It is hard and at the current stage of development of cytochemistry, it is sometimes impossible to clarify with adequate precision what the

functional significance is of the change in concentration or redistribution of various chemical substances in the cell at various stages of development of the neurons. Functional cytochemistry to a very great degree depends on functional biochemistry, which as yet has been poorly developed, and only the combined development of these contiguous trends can advance our knowledge of the rules and regulations of the functioning of the nerve cell. However, certain conclusions can be drawn even now.

RNA is changed in connection with the specific function of the nerve cells. During the course of embryonic development of the chick, when an intensive growth of neurons is occurring, the concentration of RNA in the cytoplasm is very great. However, at the turning points of the functioning of motor and sensory cells (fourth day of development of the embryo- the occurrence of specific activity of motor cells; seventh day of development- the closure of the reflex arc and the inclusion of sensory cells in the specific activity associated with this; nineteenth day- the beginning of particularly active movements of the fetus) the RNA concentration in the cytoplasm and nucleolus of these cells is altered.

The beginning of specific activity or intensification is accompanied by an increase in the RNA synthesis, which we see on the seventh, nineteenth and subsequent days of development. A reduction in the RNA concentration in motor cells on the fourth day is associated, apparently, with the fact that the reflex arc has not as yet been formed, and excitation of the motor neurons is accomplished as a result of the effect of chemical agents directly on the body of the nerve cell, that is, differently than in reflex activity. During the course of muscular work the content of protein in the motor cells of the anterior horns is considerably reduced; thereby, part of the cellular

protein is destroyed, and after this is restored by the chemical systems which form proteins (Hyden, 1943, 1955; Caspersson, 1950). At the present time, there is every basis for the belief that RNA is connected with protein synthesis (Brachet, 1957; Davidson, 1957). The recent data of Geiger (1957) are interesting; he showed that the electrical and chemical stimulation of neurons in a tissue culture produces a change in the nucleoproteins in the cytoplasm and nucleolus.

All these facts and considerations render comprehensible the increase in concentration of RNA which we observed with the intensification of specific activity of the nerve cell. In addition, it should be kept in mind that in generalizing on the data concerning the accumulation of RNA in the cell, Davidson (1957) and Brachet (1957) concluded that RNA synthesis precedes protein synthesis. In the study of fibroblastic basophilia (Levinson and Pavlova, 1949) we have also managed to show that the increase of RNA concentration in them precedes the active formation of collagen fibers. The intensification of protein decomposition associated with the onset of specific activity of neurons promptly produces an increase in the activity of the protein synthesis apparatus, which is manifested in a rapid accumulation of RNA in the cell.

Recently, K.V. Ya. Brodskiy and N. V. Nechaeva (1958ab), after using ultra-violet cytophotometry, also concluded that the quantity of RNA in the cytoplasm of ganglion cells in the retina depends on the intensity of their activity. However, it should be kept in mind that ^{the} RNA concentration in the nerve cells depends not only on the intensity of synthesis of functional protein. The embryonic neurons grow and accumulate a large quantity of protein (Hyden, 1943); finally, processes of splitting and synthesis of

protein are constantly occurring in the nerve cell associated with the general cell metabolism. Therefore, the mRNA concentration in the nerve cell is associated with its specific function, with the stage of development and the intensity of metabolism.

Along with a change in the RNA concentration at the turning point associated with a change in the intensity and nature of the specific activity of nerve cells, the concentration of the amino acids, histidine and, partly, arginine is also changed. During the course of development and adequate stimulation a change occurs in the quantity of protein in the nerve cell (Hyden, 1943; Brattgard and Hyden, 1954). Our data show that changes occur not only in the quantity but also in the quality of proteins, which is expressed in a change in the histidine concentration and partly in that of arginine in the neuron proteins.

The changes in the glycogen content with increase in the intensity of the specific activity are very characteristic. This problem has been worked out in detail with respect to nerve cells of adult animals by A. L. Shabadash (1949). Our data, obtained as a result of study of developing embryonic nerve cells, also indicate the importance of glycogen in the metabolism of nerve cells, particularly during the accomplishment of special functions by them. The functional conditioning of the redistribution of glycogen during the course of embryonic development and the concentration of it in the yolk substance only directly before hatching of the chick attracts attention and awaits explanation.

Thiol compounds are also changed in connection with a change in the neuron function. With the increase in the specific function the concentration is increased. The role ^{and} significance of ^{glutathione} /and other thiol

compounds have been analyzed well in the work of Kh.S.Koshtoyants (1951).

Hellström and Zetterström (1956), investigating retinal nerve cells, showed that as a result of adequate stimulation the concentration of SH-groups is increased. G. A. Nechayeva, N. V. Sadikova, and V. A. Skvortsevich (1957) studied the functional biochemistry of the brain by means of methionine containing S^{35} , and established the fact that the specific activity of glutathione sulfur in excited animals is, on the average, $\pm 35\%$ higher than in animals which are in a state of relative rest.

Therefore, the results obtained by histochemical methods are in agreement with biochemical data.

Conclusions

1. The basic problem of investigation^{was} the development of the functional cytochemistry of the nerve cells, that is, the detection of relationships between their structure, chemical composition and specific function.
2. A study was made of the cytochemistry of a spinal cord motor cells and the spinal ganglion sensory cells of the chick embryo at those stages of its development^{at} which changes occur in the intensity and nature of the function of the nerve cell.
3. During the course of development of the chick embryo, not only growth, change in shape and structure of the nerve cells but also a change in their chemical composition and distribution of chemical substances in them are observed. These morphological changes and changes in the chemical composition of the nerve cells are functionally conditioned.

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Cytochemical Study of Nucleic Acids in the Cells of Rabbit Brain During the
Course of Ontogeny

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It is well known that nerve cells are rich in nucleic acids (RNA and DNA), particularly RNA. The content of nucleic acids in the cells and the rate of their resynthesis change depending on the functional condition of the nerve tissue. This has been shown by numerous biochemical and cytochemical investigations on various biological objects and under various physiological conditions (Palladin, 1952, 1956; Vladimirov, 1953; Kreps, Smirnov and Chetverikov, 1954; Smirnov, 1955; Kreps and Chenykayeva, 1955; Hyden, 1955; Einarsen, 1957; Danilova, 1958; Levinson, 1958 and others). A relationship has also been established between the content of ribonucleic acid in the cell and its capacity for protein synthesis during the growth period and during functional activity (Hyden, 1943; Hochberg and Hyden, 1949; Caspersson, 1950; Brachet 1950, 1957). There are data in existence concerning the beneficial influence of nucleic acids and their derivatives on the recovery of impaired cerebral activity (Geiger and Yamasaki, 1956). At the present time there is no doubt of the fact that nucleic acids and nucleoproteins play an important part in the metabolism of the nerve cell and possibly also in the chemical dynamics of nerve processes.

A study of the content and localization of nucleic acids in the cell during the course of ontogenetic development of the central nervous system is interesting both in connection with the participation of these substances in the

processes of protein synthesis during growth and differentiation of nerve developments and in relationship with the functional maturation of the cells. The problem of this work was also the cytochemical study of the distribution of DNA and RNA in the cell structures of the rabbit brain at various stages of development.

Methods

The object of study consisted of the brains of rabbits of different ages: embryos 20, 25, 29 days; of the postnatal period, one, four, seven, 10, 12, 15, 20, 25, 30, 45, 60 days; and adult animals. The rabbits were killed by air embolism or by cutting off the head (embryos and small rabbits). Pieces three to five millimeters in thickness were excised from the cerebrum by frontal section through the motor area of the cortex; they were fixed in chilled 80 percent ethyl alcohol and embedded in paraffin.

In tissue sections (four to five microns) which were stuck to the glass slides and freed of paraffin staining reactions were carried out under strictly standard conditions which demonstrated the DNA and the RNA in the cells. Use was made of the Feulgen reaction and the Brachet method with the use of

methylgreen-pyronine and ribonuclease (Pierce, 1956). Differentiation of the tissue staining after the reaction with the stains was carried out with N-butyl alcohol, which removes the nonspecific staining with pyronine and leads to a clearer differentiation of the **methylgreen** staining (Kurnick, 1952).

Through the works of Kurnick(1947, 1952, 1955; and Mirsky, 1950, and others) it has been established that **methylgreen** selectively stains the highly polymerized form of DNA, while pyronine stains the RNA and the DNA which has not been much polymerized. Therefore, the structures containing DNA are stained

green or bluish green, and the places where RNA is localized is stained red. The distribution of RNA in the cells was controlled by a comparison of two sections, one of which was preliminarily treated with a solution of ribonuclease which specifically hydrolyzes the RNA. In the sections treated with ribonuclease staining with pyronine was usually absent. Staining of structures with methylgreen was compared with that after the Feulgen reaction, which demonstrates the DNA in the little polymerized form (hydrolysis with 1 N HCl). In this investigation parallel sections stained by the Nissl method served as the morphological control.

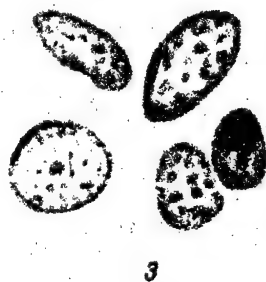
Results

The combination of the red and green staining very demonstratively shows the interrelationship of the nucleus and the cytoplasm of cells and the nature of distribution of the nucleic acids in them at various stages of the experiment (see Fig. 1,2).

The nerve tissue of 20-day-old embryos consist of fine-closely packed neuroblasts which after staining with methylgreenpyronine have homogeneous nuclei of violet color and a very small volume of bright red cytoplasm. In staining by the Nissl method the cytoplasm of these cells is indistinguishable. In sections exposed to the effect of ribonuclease, no RNA is found in the cellular elements; the cytoplasm remains colorless, and the nuclei become bluish green (Fig.3). The inhomogeneity in the intensity of staining of the nuclei becomes prominent in all the layers of the cortical plate. Part of them is stained very intensely; another part, more weakly. With great magnification (objective 120; aperture 1.30x10) small blue granules, which give the nucleus as a whole a blue color, are seen in the intensely stained nuclei against a green background. In the



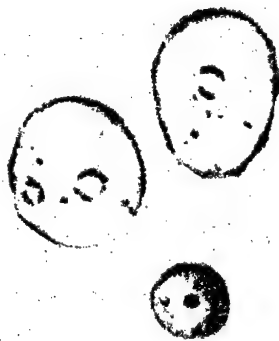
Fig. 1. Cells of cortical plate of 25-day old rabbit fetus. Staining with methylgreen-pyronine (pH 4.8). In the nuclei -- DNA and RNA; in the cytoplasm -- RNA. Distribution, diffuse. Objective, 120x; ocular, 10x.



2. Cortical cells of cerebrum of 1.5-month old rabbit. Methylgreen-pyronine staining (pH 4.8). The DNA is concentrated in the peripheral portion of the nucleoli and in the chromatin clumps; RNA is concentrated in the tigroid clumps and in the central portion of the nucleoli. Objective, 120x; ocular, 10x.



3. Neuroblasts of cortical plate of 25-day old rabbit. Staining with methylgreen-pyronine (pH 4.8), after ribonuclease treatment. The RNA has been removed. The cytoplasm has not been counterstained; the nucleus contains only DNA; the subependymal elements are richest in DNA. Objective, 120x; ocular 10x



4. Neuroblasts of cortical plate of 25-day old rabbit. Feulgen reaction. The cytoplasm has not been counterstained. The DNA is distributed throughout the nucleus, and there is a particularly large

quantity of DNA in the subependymal elements. Objective, 120x; ocular 10x.

5. Cells of cerebral cortex of 1.5-month old rabbit. Feulgen reaction. Cytoplasm not counterstained. DNA in the nucleoli, in the peripheral portion of the nucleoli and in the chromatin clumps; there is considerable DNA in the glial nuclei. Objective, 120x; ocular, 10x.

nuclei which are more weakly stained there are fewer granules of this kind. These nuclei have a pronounced greenish hue and are located chiefly in the middle portion of the cortical plate. The intensification of the greenish hue of the nuclei becomes even more prominent in the neuroblast nuclei, which form the archipallium, which, apparently, is associated with a greater degree of differentiation of these nerve elements.

The Feulgen reaction in the tissue cells of this period of development is markedly positive. The intensity of nuclear staining of the neuroblasts is similar to that with methylgreen; however, the nuclei have a more homogeneous appearance. There is always a very intense staining of the nuclear membrane.

The nerve elements, from which the archipallium is formed, are more differentiated at this time than are the neuroblasts of the neopallium. They contain a large nucleus and cytoplasm with its axon already outlined; in certain nuclei nucleoli are seen. The histochemical reactions of the nuclei of these cells are notably weaker, and the cytoplasm which has increased in size is stained intensely in the form of a fine-hardly distinguishable granulation. This indicates the fact that with the differentiation of cells the DNA content in the nucleus decreases, and the RNA content in the cytoplasm increases.

We should like to point out that the variety in intensity and hues of staining of various cellular structures is shown more markedly in the elements of the neopallium by comparison with the neuroblasts of the archipallium. Because the intensity of staining depends on the quantity of nucleic acids in the cell, and with methylgreen-pyronine staining even on the degree of polymerization of the DNA (Kurnick, 1947, 1950), it may be considered that different degrees of

stainability of the neuroblasts indicate different levels of biosynthetic activity of the cell. In the elements of the archipallium, which are more differentiated at this time but still, apparently, practically without function, the biosynthetic processes are less intense, whereby this intensity is approximately the same in all the cells. The unequal degrees of intensity of staining of the subependymal elements of all stages of development also leads us to this idea.

The ependymal and subependymal cells in the brains of the embryos give the most intense reaction both with methylgreen-pyronine and with the Schiff reagent in the Feulgen reaction. In the former case the nuclei are stained lilac (mixed color), while the rudimentary homogeneous cytoplasm is stained a bright red color. With the increase in the distance from the ependyma the staining of the neuroblast nuclei lessens somewhat, gradually changing to a brighter color in the middle portion of the cortical plate.

Beginning with the 25th day of embryogenesis a six-layered structure of the neopallium is distinctly seen. Beginning with this time and up to the end of the embryonic period (30 days) there is a rapid increase in the size of the cell body, particularly in the V layer and in the structures of the archipallium. Here, the nature of stainability of the cells with methylgreen-pyronine is changed. The diffuse coloration of the nuclei becomes progressively brighter and greener. Nucleoli and chromatin clumps are seen in them more distinctly. In the cytoplasm there is an increase in the number and size of the granules which are stained with pyronine. All this is less pronounced in the upper cortical layers, where differentiation is delayed. During the course of the first four days after birth of the rabbit a bichromatic coloration of the

nucleoli is distinctly seen in all the cells: red (RNA) in the central portion and green (DNA) along their periphery. However, the karyoplasm still has a mixed type of staining, which disappears between the eighth and 15th day of the animal's life and is maintained longest in the II layer of the cortex.

The ~~neurons~~^{neurons} of the adult animal have a cytoplasm containing a high RNA content, which is included in the tigroid clumps, and a nucleus with various chromatin granules containing DNA. In the nuclei one or two bichromatic nucleoli are very distinct.

The picture of staining of the nucleolus in our preparations is in complete agreement with the distribution of its chemical components according to the data of investigations of ^{the} Caspersen school; he established the fact that the nucleolus contains a large quantity of RNA surrounded by clumps rich in DNA at the periphery. The representatives of this school believe that the nucleolus and the chromatin associated with it represent an active portion of the nerve cell during protein synthesis (Hyden, 1943, 1955).

In the elements of glial tissue of the grey and white matter of the cerebrum of adult animals an intense green staining of the nuclei with small, also bichromatic, nucleoli, is seen with staining by the Brachet method. The glial cytoplasm is small in volume and poor in RNA. The nuclei of the microglia are always more compact and richer in their DNA content than the nuclei of the macroglia. In accordance with this they give a very positive Feulgen reaction. The mature ~~neurons~~^{neurons} usually give a weak nuclear reaction. After treatment with the Schiff reagent only the nuclear and nucleolar outlines and various chromatin granules have a red color (Fig. 5).

From what has been presented it is seen that the nuclei of neuroblasts

as well as the nuclei of glia of mature tissue are richer in their DNA content than the nuclei of neurons. The RNA content, on the other hand, is greater in mature neurons.

According to the morphological data of A.S. Pentsik (1937), A.S. Troitskaya (1953) and A.M. Ivanitskiy (1958 a, b, c), differentiation of the inner structure of the neuron body was completed by the 10th-15th day after the birth of the rabbit. By this time the chromatin substance of the cell nuclei changes from a diffuse state to a state of concentration in the various structures. However, this process like the birth of the cell body, is finally concluded by the age of one month. This is reflected also in the cytochemical reaction.

Beginning with the middle of the first month of life of the rabbit the nature of the reactions for nucleic acids change little. Only certain differences are noted in the intensity of staining of various cells in all the cytoarchitectonic layers. They are expressed either in a slight increase in the stainability of the karyoplasm of certain cells or in the great accumulation of the granular substance in the cytoplasm near the nucleus, at the place where the dendrites come off.

Therefore, it may be said that during the course of cytochemical maturation of the nerve elements the nature of distribution of nucleic acids and nucleoproteins changes. From a diffuse distribution in undifferentiated elements they are organized in corresponding structures of the mature nerve cell. Here, the quantity of DNA in the nucleus decreases considerably, while the quantity of RNA in the cytoplasm is increased, gradually reaching a level characteristic of cells of the mature animal.

Discussion

It is interesting to compare our observations with the biochemical data obtained on the same biological object. It has been established through biochemical analyses that the content of DNA and RNA in the brain tissue is high at the early stages of development. It gradually decreases by the end of the embryonic period, continues to decrease, although more slowly, after birth, and by the age of one month reaches a level characteristic of the adult rabbit. These rules and regulations were shown in works on the entire rabbit brain (Skvirskaya and Chapinova, 1953; Skvirskaya and Silich, 1954) and in its sections (Manukyan, 1955 a, b). Manukyan showed that in the cerebral cortex the content of DNA is higher on the 20th-23rd day of embryogenesis than that of RNA. The DNA and RNA content are equal on the 26th day of embryonic development. In the adult rabbit the quantity of DNA is nine times less and that of RNA, 2.5 less than in the 20-day-old embryo. The cytochemical data are in complete agreement with this. In comparing microscopic pictures as well as the intensity of the cytochemical reactions for DNA and RNA in the embryonic tissues and the tissues of adult animals the difference is graphically seen in the content of these substances in the cells. In the embryonic tissue the cell nuclei are rich in DNA; they are small but compact. In the mature cortex, on the other hand, the nuclei are large, scattered, and poor in DNA. Because of this the quantity of DNA ^{per} unit volume of mature tissue is considerably less.

The coincidence of cytochemical and biochemical data also well illustrates the interrelationship of the RNA and the DNA at various periods of development. According to our data, the quantity of RNA increases with the growth of the cell, while the quantity of DNA decreases. Therefore, the quantity of RNA

should increase per unit of DNA in the nucleus. Manukyan has shown that the ratio RNA/DNA actually changes markedly: in the 20-day-old embryos it is equal to 0.69, while in the 30-day-old rabbits, to 2.8. In biochemical investigations the total mass of tissue was used; therefore, the data are average. Our microscopic observations show that the ratio RNA/DNA is different in different groups of nerve cells. Thus, for example, in a large pyramidal cells of the V layer of the cerebral cortex of rabbits it is considerably greater than in the small pyramidal cells of the II-III layers. An altogether different relationship occurs between the RNA and DNA in the glial elements, which are rich in DNA and poor in RNA.

V. Ya. Brodskiy (1956) has established the fact by the method of cytophotometry that the quantity of RNA in the ^{neu}uron depends on the size of the neuron. The greater the size of the cell body the higher the content of RNA. Therefore, with slight variability in the DNA content the ratio RNA/DNA is in correlation with the size of the cell and apparently is associated with the functional significance of cells of different dimensions. The data of Edström (1956), who found that cells of the same narrow functional group have similar volumes and similar contents and concentrations of RNA, serve as confirmation of this.

All the data on the cytochemical, morphological and functional maturation of the cerebral cortical cells show the close interrelationship of the chemistry, structure and function. The chemical changes lead to the occurrence of structures needed for the accomplishment of the function, while the function, in its turn, exerts an influence on the further development of the structure and chemistry.

Hyden (1955) indicates that physiological stimulation is required for the normal post-natal development of cells. If there is no physiological effect at the corresponding stage of development the chemical and functional maturation of cells stop.

The morphological and functional maturation of nerve cells is indivisible from processes of protein synthesis, which the nucleic acids undoubtedly play an important part. The high content of DNA and RNA in the cells at different stages of ontogeny, wherein an active growth and differentiation of cellular elements occurs, is in complete agreement with this. The DNA apparently plays an important part during the period of division in the original growth of the cells. The role of RNA judging by its accumulation in the cytoplasm, is changed with the age of the cell: apparently, the RNA is also included in providing for the specific function of the nerve cell.

From this point of view it is interesting to compare the change in the content of DNA and RNA during the course of development of nerve cells with changes in the activity and distribution of alkaline and acid phosphatases -- enzymes which are related to nucleic acid metabolism.

During the early periods of development of cellular elements the activity of the alkaline and acid phosphatases were shown cytochemically diffusely in the nuclei, and the cytoplasm remained indistinguishable because of its small volume. In the mature neurons the alkaline phosphatase was found in the cytoplasm; also in the nucleus, chiefly in the nucleolus; the acid phosphatase, in the nucleus, in the clumps of different sizes and in the nucleolus (Krasil'nikova, 1938). Kabinovich (1949) also found that there is a high degree of activity of the acid phosphatase in the particles constituting the periphery of the

nucleolus (rich in DNA) and of the alkaline phosphatase in the nucleolus proper of the nerve cells of the guinea pig.

According to the data of biochemical investigations (Chirkovskaya, 1956), the acid phosphatase in the cerebral cortex of the rabbit has the greatest activity at the early stages of development during the course of ontogeny. This coincides with the highest content of DNA in the nerve tissue during the period of the highest activity of the cell nucleus. No relationships are found between the changes in activity of the acid phosphatase during the course of ontogeny and the functional development of the nervous tissue. On the other hand, a complete agreement is found between the activity of the acid phosphatase and the uptake of P^{32} in the DNA in various portions of the brain and in the various periods of ontogeny (Manukyan, 1955a, b; Chirkovskaya, 1956).

Changes in the activity of the alkaline phosphatase coincide in time with the functional maturation of the cerebral cortex. The parallelism in the localization of alkaline phosphatase and RNA in the cell cytoplasm as well as the relatively high degree of the enzyme ^{activity} and the high RNA content in the cytoplasm and nucleolus of the mature nerve cells can indicate a relationship between the alkaline phosphatase and the RNA metabolism and the participation of these substances in the accomplishment of the specific nerve cell function.

Conclusions

1. A study was made of the content and distribution of nucleic acids in the cells of the cerebral cortex of the rabbit brain during the course of ontogeny by cytochemical methods. It was established that during the course of cytochemical maturation of the nerve elements the nucleic acids and nucleoproteins,

which are diffusely distributed in the neuroblasts in the early period of embryogenesis, gradually become localized in the corresponding structures of the mature neurons.

2. The content of DNA is high in the early stages of development and decreases considerably by the time of maturation of the nerve cells. The RNA content, on the other hand increases with the growth of the cell body, and it is greater the larger the volume of cytoplasm.

3. Cytochemical observations, in complete agreement with biochemical data, indicate the participation of DNA in the processes of original growth and differentiation of tissue, providing for its further development. RNA, aside from its participation in the growth processes, apparently takes part at later stages of development in providing for the specific nerve cell function.

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Cytochemical and Autoradiographic Investigation of the Seasonal Changes in

Amphibian Ova

(Presented at the conference in memory of the Academician A.A. Zavarzin 16 April 1959)

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Modern methods of cytological analysis make it possible to evaluate the processes occurring in cells, which previously had escaped the attention of research workers, and also make it possible to interpret more accurately the pictures observed. What has been stated applies in full measure to amphibian oocytes, which for a long time now have been one of the favorite biological objects of study and which to date present quite a little interest from different points of view.

In carrying out the present work use was made of/natural "experiment", which has been refined by thousands of years, seasonal changes. In the literature devoted to the characteristics of the structure and development of ova of organisms with complex life cycles, such as amphibians, the proper attention has never been given to the relationship of these processes and the period of the year. Not uncommonly, a study was made of an "abstract" frog, and on this basis conclusions were drawn, for example, those dealing with yolk-formation, the behavior of certain nuclear/structures, etc. At the same time, an abundance of data obtained chiefly by physiologists speaks for the fact that frogs are different in a number of essential characteristics and properties in the winter, spring and summer.

Material and Methods

The object of study consisted of ova of the frog Rana temporaria L. at different seasons of the year and of different ages. In the spring, summer, and autumn the animals were caught in their natural habitats; in the winter they were brought to the laboratories directly from their places of hibernation. As far as possible the material was taken immediately for fixation, or experiments were performed with the administration of isotopes. (This section of the work was carried out in the Laboratory of Histology of the IEM AMN SSSR /Institute of Experimental Medicine of the Academy of Medical Sciences USSR, headed by Professor L.N. Zhinkin).

At the present time, the method of tagged atoms has become widespread for the purpose of investigating many biological processes, including also for the purpose of evaluating the metabolic rate of substances and the course of its various phases. In cytology autoradiography has opened up great possibilities, making it possible to study simultaneously cell structures and the uptake of various isotopes in them. This method makes it possible to demonstrate changes in the functional properties of cells which previously were undetectable.

In this work use was made of S^{35} -methionine and P^{32} -sodium phosphate solutions of which were injected into the spinal lymph sack in all cases in a dose of two microcuries per gram of weight of the animal. At various intervals after the injection of the isotope (from three hours to 30 days) the ovaries were removed and fixed with Bouin's fluid and 10 percent formalin acidified with several drops of acetic acid. The embedding was accomplished with terpinol and paraffin. A light-sensitive emulsion (in accordance with the instructions of the NIKFI /Motion Picture and Photography Scientific Research Institute/)

was applied to the deparaffined sections. After different exposure times (from one to 16 days) the developing and preparation of the autoradiographs were performed.

In those areas of the oocytes in which the isotope under study was taken up black lines were found -- tracks or traces of electron pathways. The autoradiographs were counterstained with alum-carmin and 0.1 percent aqueous solution of methylgreen which produced a good color contrast and did not stain the emulsion.

Along with the autoradiography use was made of various cytological, including cytochemical, methods. In the present work, the results of staining preparations for ribonucleic acid (RNA) with methylgreen pyronine according to the Unna and with eosin-azure II according to the Maximow methods (the control material was exposed to the effect of ribonuclease and hydrolysis and 1N HCl at 60° for five minutes) were used as well as those attained with fast green (pH 2.2 and pH 8.5) for the purpose of demonstrating the total and the basic proteins.

The pictures observed were delineated by means of a drawing apparatus and were also photographed.

The number of nucleoli per nucleus was calculated in serial sections by means of an ocular grid. Only nucleoli more than two microns in diameter were taken into consideration.

Uptake of Isotopes in Oocytes

The uptake of tagged amino acids in the oocytes of the frog was investigated by Ficq (1955), who apparently performed his experiments in the late winter or early spring. Ficq notes that the synthesis of protein proceeds most actively in oocytes of average size, whereas the largest ova take up amino acids

only into the nucleus and relatively slowly at that. The autoradiographic investigation of amphibian oocytes was carried out also by Kemp (1955) and Pantelouris (1958).

The absence of radioactive sulphur in the yolk bodies of birds has been noted by Shieh Sher-pu and Pu I-sen (1958).

According to our data, S^{35} -methionine is taken up by the nucleus and cytoplasm of oocytes of average size both in the case of adult animals and in two-year-old frogs very actively during the summer (June) and approximately at the same rate (Fig. 1a), that is, in those ova which reach the state of maturity the following spring. These oocytes grow in an active manner, and the process of vitellogenesis proceeds in them in a lively manner. The uptake of tagged amino acids occurs much more slowly (Fig. 1,b) in the small oocytes of the same animals, and, particularly, in the oocytes of the one-year-old frog. Therefore, the synthesis of cell proteins occurs most actively in those oocytes which can become ready for the ovulation period during the summer (Table 1).

In the oocytes of medium size in adult and two-year-old frogs the uptake of P^{32} -phosphate occurs actively also. Tagged phosphorus in the period under investigation (May) is detected chiefly in the karyoplasm and in a much smaller quantity in the cytoplasm (Fig. 4,a). P^{32} is taken up chiefly in the phosphoproteins, which is indicated by the results of removal of nucleic acids, desoxyribonuclease, ribonuclease as well as by the treatment of sections with five-percent trichloroacetic acid (90 percent, 15 minutes): after this, the number of tracks of P^{32} on the autoradiographs does not change in any notable fashion. The presence of protein phosphorus in the cytoplasm and particularly in the karyoplasm has been confirmed cytochemically (treatment by the Serr and Lopez method; see Roskin and

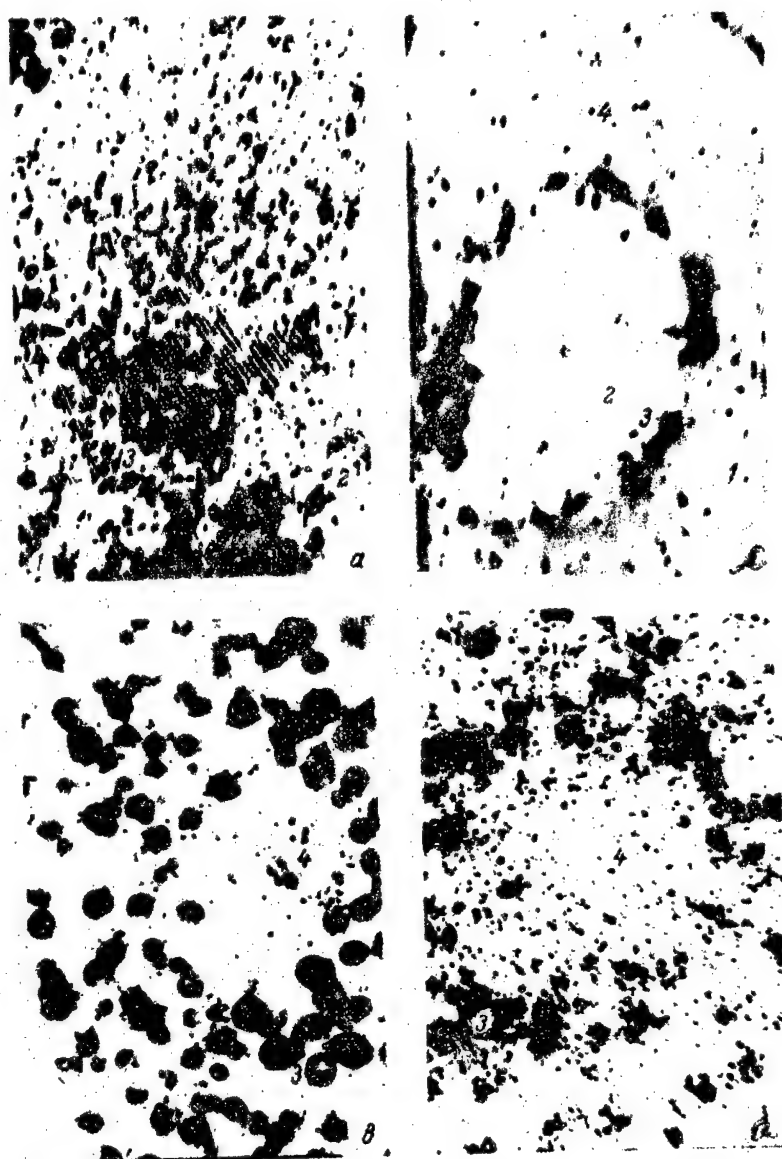


Fig. 1. Uptake of S³⁵-methionine in frog oocytes.

- a) growing oocyte of sexually mature frog, June, six hours after injection of isotope;
 - b) oocyte of one-year-old frog, same experiment as in a;
 - c) part of nucleus of large oocyte, October, six hours after injection of isotope;
 - d) same, 72 hours after injection of isotope.
- 1 - cytoplasm; 2 - nucleus; 3 - nucleoli; 4 - tracks of S³⁵. Exposure time, 4 days; preparations counterstained with alum-carmin. a & b - magnification 300x; c & d - magnification 420x.

Levinson, 1957).

At the end of September to the beginning of October the production of cytoplasmic structures of large oocytes is basically completed. These oocytes are filled with yolk granules; they contain pigment which marks the animal pole. S^{35} -methionine is taken up in their cytoplasm in a negligible quantity, that is, the protein synthesis does not occur to any notable extent in it. At the same time, the production of nuclear proteins continues in those oocytes, and quite actively. This can be judged by the fact that the S^{35} tracks are found in the nuclei as early as six hours after the injection of the isotope (Fig. 1,c). The content of tagged amino acid in the karyoplasm increases with time (Fig. 1, d).

The same picture, in principle, that is, the uptake of S^{35} -methionine chiefly in the nuclear proteins, is observed in large oocytes even in the subsequent months (November-April). However the rate of uptake of the amino acid understudy during the winter months decreases sharply. Thus, in November, four days after the injection of the isotope it can not yet be detected in the nuclei (Fig. 2,a); this can be done only on the seventh-11th day after the injection (Fig. 2,b). Thirty days after the injection the accumulation of S^{35} -methionine in the nuclei is very considerable (Fig. 2,c); therefore, the separation of tagged sulphur from the nuclear proteins, if it occurs, is on a very limited scale. Such a low level of nuclear protein synthesis is maintained even in the subsequent months.

Therefore, during the winter, beginning with October, practically no increase occurs in the mass of the cytoplasmic proteins in the large, already formed oocytes, which is understandable if we take into consideration the fact that vitellogenesis at this time has been completed. At the same time, at this time the completion of the construction of nuclear proteins, apparently those which are primarily included in the nucleoli, is still proceeding. The intensity of this process decreases from month to month (Table 2).

Table 1

Intensity of Uptake of S^{35} -Methionine on Oocytes of the Frog Depending on Their Age (June, 6 Hours After Injection of Isotope, Exposure Time 3 Days).

1) Возраст лягушки	2) Количество измеренных ооцитов	3) Диаметр ооцитов (в мк)	4) Рассчитанная площадь (в тыс. мк ²)		5) Число треков на 100 мк ²	
			6) Ядро	7) Цитоплазма	8) Ядро	9) Цитоплазма
Однорезки . .	25	200—250	62.5	62.5	1.76	1.29
Двухрезки . .	10	600—650	125	125	4.92	4.68

- 1) Age of frogs; 2) Number of oocytes measured; 3) Diameter of oocytes (in microns); 4) Area calculated (in thousands of square microns); 5) of nuclei; 6) of cytoplasm; 7) Number of tracks per hundred square microns; 8) nucleus; 9) cytoplasm.

Table 2

Intensity of S^{35} -Methionine Uptake in Already Formed Oocytes of the Frog During the Autumn-Winter Period (Number of Tracks per 100 Square Microns with an Exposure Time of One Day)

① Месяцы	② Количество измеренных ооцитов	③ Рассчитанная площадь (в тыс. мк²)		④ Время после введения изотопа									
		④ ядро	⑤ цитоплазма	6 мес		24 мес		72 мес		11 days		30 days	
				ядро	цитоплазма	ядро	цитоплазма	ядро	цитоплазма	ядро	цитоплазма	ядро	цитоплазма
Октябрь .	10	250	125	1.76	0.52	2.88	0.48	5.96	0.64	—	—	—	—
Ноябрь— декабрь .	10	250	125	0	0	0	0	0	0	2.40	0	4.64	0
Январь— февраль .	10	250	125	0	0	0	0	0	0	2.72	0.14	1.96	0.20

- 1) Months; 2) No. of oocytes measured; 3) Area calculated (in thousands of square microns); 4) of nuclei; 5) of cytoplasm; 6) Time after injection of the isotope; 7) October; 8) November-December; 9) January-February.

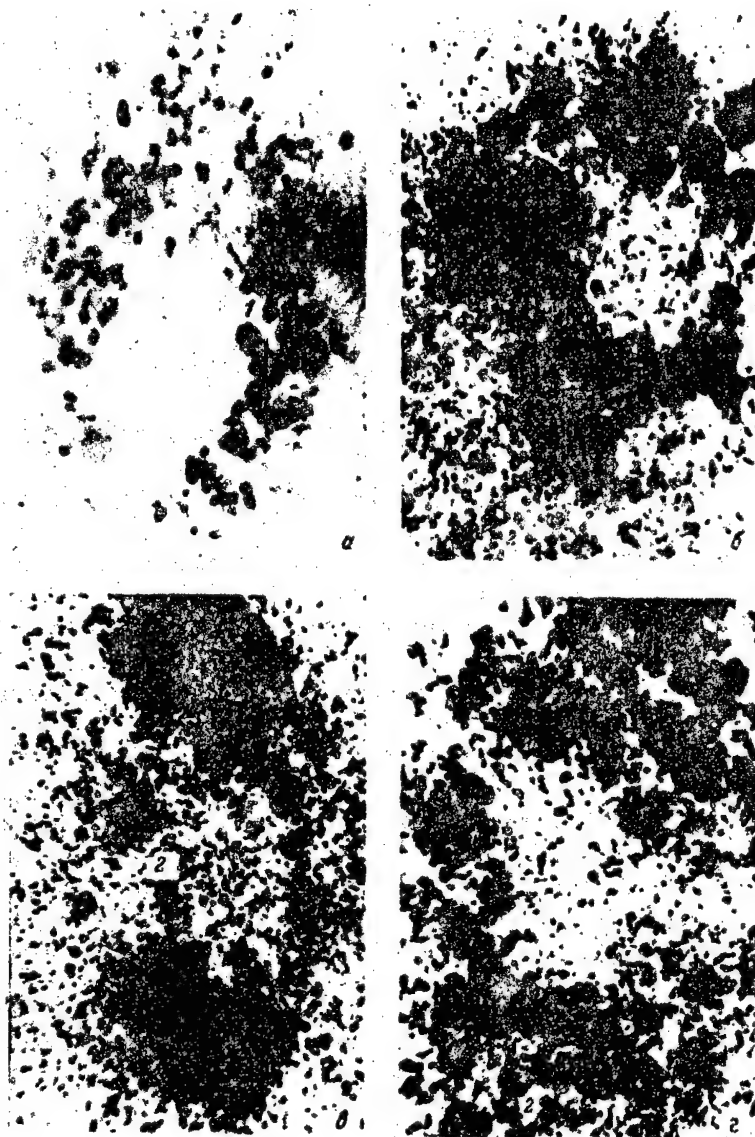


Fig. 2. Uptake of S^{35} -Methionine in the nuclei of large frog oocytes. The central portions of the nuclei are seen on the microphotographs.

a) November, four days after the injection of the isotope; b) the same, 11 days after the injection of the isotope; c) the same, 30 days after the injection of the isotope; February, 30 days after the injection of the isotope. 1) Nucleoli; 2) S^{35} tracks. Exposure time, 4 days, preparation stained with alum-carmin. Magnification: 42x.

In the same season, beginning with October, S^{35} -methionine stops being taken up in all the remaining moderate-sized and small frog oocytes of all ages (Fig. 3,a, b), or it is taken up very slowly. As a rule, the tracks of S^{35} are absent from the cytoplasm and from the nucleus any interval, even long ones, following the injection of the isotope. In other words, these isotopes go into a state of "anabiosis", so to speak, or a "diapause". Such a reaction on the part of these oocytes is understandable from a biological point of view: if their growth continued in the winter, they would not be able to reach the proper degree of maturity at the time of spawning, being unable to pass through the appropriate stages of development during the summer period, and would be subject to massive atresia. Incidentally, in the spring such oocytes at various stages of destruction are quite frequently encountered.

The diapause of the moderate-sized and small oocytes last from October to the end of January-beginning of February. Beginning with this period (here individual variations are observed) S^{35} -methionine begins to be taken up intensely in the cytoplasm and nuclei (Fig. 3,c), that is, the synthesis of cell proteins is renewed and the growth of oocytes begins again. The fact should be emphasized that

elimination of the diapause occurs in animals which have not received nutrition and which are still in the typical "winter" conditions.

The intense uptake of S^{35} -methionine in oocytes of average size, beginning with January-February, occurs also in subsequent months (Fig. 3,d), reaching a maximum in the summer period. During this time, considerable growth

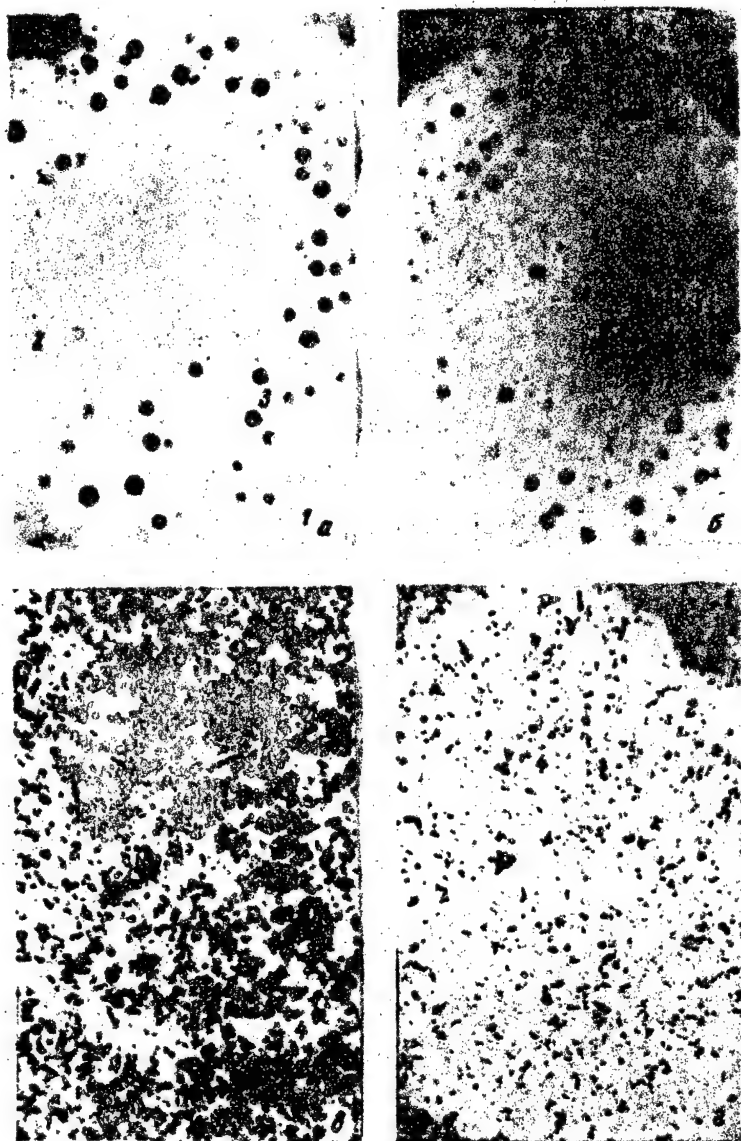


Fig. 3. Uptake of S^{35} -methionine in the oocytes of average size.
 a) October, 72 hours after injection of isotopes, No S^{35} tracks; b) November, 30 days after the injection of isotopes, No S^{35} tracks; c) February, 30 days after the injection of the isotope, intense uptake of S^{35} in the cytoplasm and nucleus; d) April, six hours after injection of the isotope, S^{35} uptake in cytoplasm and nucleus. 1) cytoplasm; 2) nucleus; 3) nucleoli; 4) S^{35} tracks. Exposure time four days, preparations counterstained with alum-carmin. Magnification, 200x.

occurs in them, and they are converted into large, well formed ova.

Later, in October, all the phenomena of which we spoke above unfold in them.

During the winter the uptake of P^{32} -phosphate in the oocytes of all sizes stops completely (Fig. 4, b, c). Tagged phosphorus cannot be detected either in the cytoplasm or in the nucleus.

Thus, when the method of autoradiography has been used interesting seasonal and age changes in the intensity and distribution of cell protein synthesis in the are demonstrated and, therefore, also growth of the frog oocytes. In the large, well-formed oocytes the formation of proteins in the cytoplasm stops almost completely by October. However, the increase in the mass of the nuclear proteins, including the nucleoli, occurs during the winter months (November-January) on a decreasing scale. The average and small oocytes change into an inactive state (diapause) beginning with the end of September-beginning of October, whereby the synthetic processes either stop or proceed extremely slowly. Such a condition lasts until the end of January-beginning of February, when the oocytes of this size renew their growth.

in the Changes in the Nucleoli and/RNA Content

How are the changes in protein synthesis of the oocytes under analysis reflected in the content of nucleic acids in them? It is well known that at the present time the reproduction of proteins is associated with nucleic acids (Caspersson, 1950; Allfrey and Mirsky, 1957; Allfrey, Mirsky and Osawa, 1957 and others). In the present work, we will deal only with RNA. The problem of the

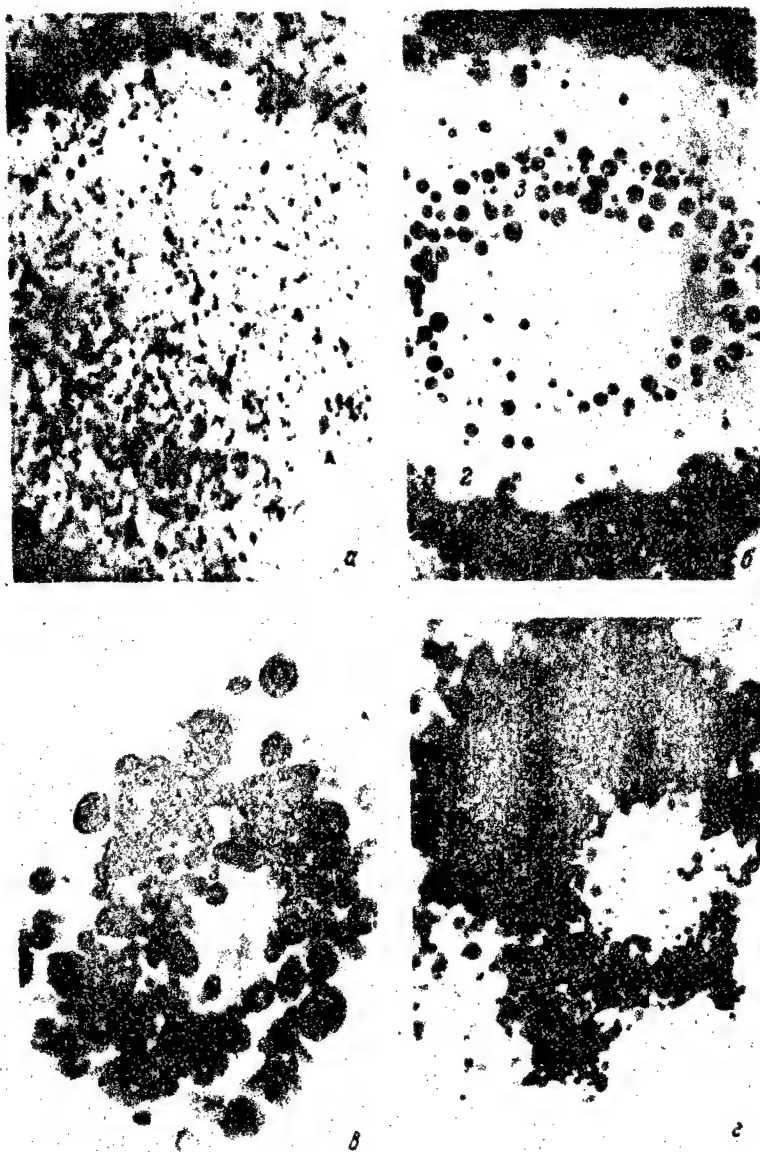


Fig. 4. Uptake of isotopes in oocytes of the Frog.

a) growing oocyte, May, P^{32} -phosphate, 72 hours after injection of isotope; b) oocyte of the same size, November, P^{32} -phosphate, 96 hours after injection of the isotope; c) section of nucleus of large oocyte, November, P^{32} -phosphate, 96 hours after injection of isotope; d) section of nucleus of large oocyte, November, S^{35} -methionine, 30 days after injection of isotope, focussing on tracks located over the nucleoli. 1) cytoplasm; 2) nucleus; 3) nucleoli; 4) S^{35} or P^{32} tracks. Exposure time four days. The preparations are counterstained with alum-carmin. a and b) magnification 300 x; c and d) magnification 420x.

interrelationship between proteins and desoxyribonucleic acid of the nuclei of amphibian oocytes (Makarov, 1958, 1959) deserves separate analysis in a special article.

First of all, it is necessary to become acquainted with the changes in the nucleoli of the nuclei of large oocytes. The fact attracts attention that after the completion of vitellogenesis the number of nuclei increases precipitously (compare Fig. 5, a with Fig. 5, c). Thus, in September there is an average of 201 nucleoli; in October, 878; in December, 1236. The maximum number of nucleoli is observed in January, 1473 per nucleus. In other words, during the period of September-January the number of nuclei increases by more than seven times. The size of the nuclei during this period of time remains more or less stable; therefore, in this case a steady increase in the mass of the nucleolar material occurs.

These observations on the synthesis of the nucleolar substance are in full agreement with the data of autoradiography (Fig. 4, d). The tracks of ³⁵S-methionine are located chiefly around the nucleoli. In this area there are one and one-half to two times more of them than in the other areas of the nucleus. Such a phenomenon was also noted by Ficq (1955) and Pantelouris (1958).

In the nuclei of the large frog oocytes there are apparently two generations of nucleoli. During the period of vitellogenesis the nuclei lie at the periphery of the nuclei, directly under the nuclear membrane (Fig. 5a). They are markedly vacuolated, rich in RNA and acid proteins, as a rule; there are few basic proteins in them and sometimes there are none at all, that is, after

staining with fast green at pH 8.5 the nucleoli may remain colorless.

In preparations stained according to the Unna method it is possible to observe pictures which constitute evidence of the dissolution, and destruction of such nucleoli: unevenness of the outlines, stainability with different degrees of intensity up to preservation of only pale shadows, etc. In October oocytes are encountered in which part of the nucleoli, larger and variously stained, are located at the periphery of the nucleus; others, smaller ones, lie in the center of the nucleus (Fig. 5b), forming in their totality a kind of ring, inside which there are chromosomes. Beginning with November the nucleoli are localized exclusively in the center of the nucleus; there are none of them at the periphery (Fig. 5c). The vacuoles detectable in such nucleoli are relatively few; the nucleoli are rich in RNA and basic proteins. Therefore, the nucleoli of different locations, peripheral and central, are distinguished from one another not only by their location but also by their chemical composition. All these data permit us to suppose the existence of two independent generations of nucleoli. However, this conclusion still requires checking and further argumentation. As a matter of fact, sometimes pictures of displacement of the large peripheral nucleoli toward the center of the nucleus are encountered. The degree to which this phenomenon is a regular one should be clarified by further investigations.

We should emphasize the fact that the increase in the mass of nucleoli occurs after the synthesis of cytoplasmic proteins stops in the oocytes, which we can judge reliably by the autoradiographic data. This observation contradicts the assertion made by Caspersson (1950), according to whom an increase in the mass of nucleoli is characteristic of cells with an active protein synthesis. In the oocytes of the frog the opposite phenomenon occurs: during

the period of formation of the yolk the nucleoli are relatively few and show signs of dissolution; after vitellogenesis stops the mass of the nucleoli increases considerably.

From the end of January to the beginning of February the number of nucleoli in the nuclei of large oocytes decreases somewhat because of a degeneration and apparently a dissolution of them. This process begins with the nucleoli located in the peripheral area of their central accumulation. Here, an infinite number of very small nucleoli -- "nucleolar dust", is found. The reduction or even

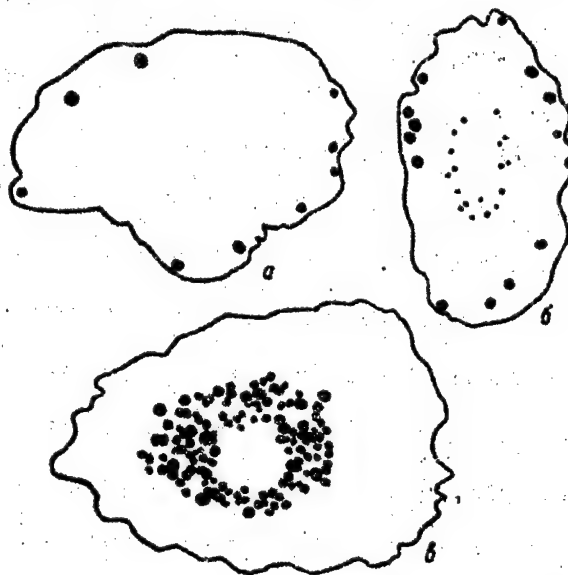


Fig. 5. Seasonal changes in the number and location of nucleoli in the nuclei of large oocytes of the frog.

- a) September, nucleoli few; located at periphery of nucleus;
- b) October, appearance of small central nucleoli;
- c) December, nucleoli are many; they are located in the center of the nucleus. Fixation according to the Helly method; staining according to Unna method; magnification, 70x.

cessation of protein synthesis in the nucleoli during this period is confirmed by the results of autoradiography (Fig. 2,d).

It is very difficult to evaluate reliably the seasonal changes in the nucleoli of the nuclei of ^{moderate-sized} / and small oocytes. Here, they are often very small and their location is changeable. Further investigations are needed to show any essential rules and regulations.

A reduction in the basophilia of the cytoplasm of the oocytes during the course of vitellogenesis has been noted by many authors. This phenomenon is usually explained (Brachet, 1947; Fautrez--Firlefyn, 1951, and others) by the distribution of a constant quantity of RNA in an increasing volume of cytoplasm, that is, it is considered only apparent. At the same time, a number of research workers does not share this viewpoint and describes the consumption of RNA during the course of formation of the yolk (Pavlova, 1952, 1959a; Durand, 1952; Petrova, 1956; Chubareva, 1957). It seems to me that references to an increase in the volume of the cytoplasm as the cause ~~for~~ of only an apparent reduction in the RNA content is unfounded on an a priori basis. As a matter of fact, during the period of considerable growth, that is, vitellogenesis, the quantity of the cytoplasm proper does not increase, while the increase in the size of the oocytes proceeds through the accumulation of the deutoplasm -- yolk granules -- in them.

During the course of oogenesis in the frog pictures are observed which evidently speak for a loss of RNA during the course of vitellogenesis. Before the onset of this process considerable RNA is present in the cytoplasm of the oocytes, and it is distributed uniformly throughout the body of the cell. With the formation of yolk granules, which begins from the periphery and spreads to the center of the oocytes, a disappearance of the basophilia is observed which

had been produced by the presence of RNA. RNA is found in a progressively decreasing quantity in the perinuclear and cortical areas of the oocytes which are free of yolk. A marked reduction in the content of RNA during the course of vitellogenesis may not be accompanied by any considerable increase in the size of the oocytes at first. Afterwards, when the yolk granules fill the entire cytoplasm the RNA can no longer be detected in it.

How does the content of cytoplasmic RNA of the average oocytes change during the "diapause", that is, during the period when, according to the autoradiographic data, the synthesis of cell protein stops? The cytoplasm of such oocytes possesses an active basophilia; in other words, considerable RNA is present in it. Moreover, basophilia at this time is greater than at the height of vitellogenesis. Thus, in November, the pyroninophilia of the cytoplasm is approximately eight times more intense than in the oocytes of the same size in July. (The intensity of pyronine staining was evaluated according to a ten-plus scale with the use of special standards and a comparator microscope).

Therefore, in spite of the very widespread opinion (Brachet, 1947; Caspersson, 1950; Tsanev, 1951; Tranev, 1958 and others), the richness of the cells in RNA does not in itself speak for the fact that a process of intense synthesis of proteins is occurring. Rather, the high content of RNA attests to the opposite, that is, a decrease in the synthetic activity of cells. Cells rich in RNA are capable of beginning protein synthesis under the proper conditions; they constitute a kind of charged storage battery. During the course of this synthesis the RNA is used up (Pasteels, 1948; Makarov, 1955; Puchkov, 1959, and others). It serves as a source of specific energy necessary for building cytoplasmic proteins (Spiegelman and Kamen, 1947). However, the energy reserve

accumulated in the cell in the form of RNA rich in phosphate bonds does not have to be used up right away, which is seen at least through the example of the ova of flat worms (Bogomolova, 1959; Pavlova, 1959b).

RNA is used up in the course of synthesis of cytoplasmic proteins, but at the same time as it is used up a process of RNA resynthesis may also occur. Usually, the formation of cell proteins, especially when it is proceeding rapidly, involves a reduction in the content of RNA in the cell, but this phenomenon may not be observed in the event the rate of consumption of RNA corresponds to the intensity of the process of resynthesis of it.

Conclusions

1. During the spring-summer an active uptake of S^{35} -methionine and P^{32} sodium phosphate occurs in the cytoplasm and nucleus of large and moderate-sized oocytes of adult frogs as well as those of two-year-old frogs. In the small oocytes of these animals and in the oocytes of the one-year-old frog the uptake of isotopes occurs more slowly.
2. Beginning with October, after the completion of vitellogenesis in the cytoplasm of large oocytes, the S^{35} -methionine is found only in a negligible quantity. The synthesis of nuclear proteins continues throughout the entire winter, but its intensity decreases from month to month.
3. Beginning with October, the small and moderate-sized oocytes go into a state of reduced activity (diapause). At this time, the tagged amino acid is not taken up at all in the cytoplasm / or in the nucleus or is taken up very slowly. The growth of oocytes begins again with the end of January-beginning of February, when the isotopes administered are again found in them.

4. P^{32} sodium phosphate is not taken up in the oocytes of any size throughout the entire winter.

5. After the conclusion of yolk-formation the number of nucleoli in the nuclei of large oocytes considerably increases. Probably, two generations of nucleoli are formed in these nuclei which are different from one another in their locations as well as in their chemical composition.

6. During the diapause, when the synthesis of cell proteins stops the content of RNA in the cytoplasm of the moderate-sized oocytes is greater than in the summer, ^{the} / ^{quantity} / active period. A large / of RNA in the cells cannot serve as an index of a high degree of intensity of protein synthesis in them.

7. A number of data permits us to assert that RNA is used up in the course of synthesis of cell proteins: it apparently serves as a source of energy for accomplishment of this process.

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Mechanisms of Autoreproduction of Elementary Cell Structures

I. From the History of the Problem

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1. Life on earth is represented by numerous forms of living organisms which are characterized by having metabolism and energy (associated with the ability to synthesize specific organic macromolecules), growth and multiplication. Part of the organisms (if we include viruses and phages among them) possess an exceptionally simple, acellular structure; the others are either free-living or parasitic cells (or simple aggregates of cells) or are, to various degrees, complex and differentiated multicellular individuals. Sexual multiplication is characteristic of the majority of living organisms, whereby union of two sex cells--gametes -- into a single cell -- the zygote -- leads to the formation of individuals of the next sexual generation. A characteristic feature of the multiplication of living organisms is reproduction of individuals of each form with all characteristic morphological and physiological features. From this, the necessity follows for recognizing the presence of a complex regulating system in cells of every form of living organism and a kind of code of hereditary information which is transmitted from generation to generation. This code should be represented by a material substrate in the cells and should possess a relatively high degree of stability and the ability of autoreproduction.

2. Beginning with the middle of the 19th century a careful microscopic study of the phenomena of cell division, maturation of gametes, fertilization and ontogenetic development of the zygote, was begun. By the end of the past and the beginning of the present century the outstanding mechanisms of ~~mitosis~~ mitosis and meiosis, which were of the same form in principle and characteristic of all organisms, had been studied in their main outlines in the classic works of a whole series of biologists (for example, Strasburger, 1882, 1894, 1905; Boveri, 1887, 1904, 1909; R. Hertwig, 1888, 1902; Belajeff, 1894; O. Hertwig, 1900; Sutton, 1902; McClung, 1902; Navashin, 1899, 1914). Here, the discovery of special nuclear organelles -- chromosomes -- which, as a rule, are present in every form of living organism in a very definite number of pairs, possess typical size and structure, and undergo reduction during meiosis as a feature common to all cells, was most surprising. Even in the cytoplasm of cells there are structural formations (mitochondria, plastids, etc.) which apparently possess the capacity of autoreproduction, like the chromosomes, but the chromosomes are undoubtedly most carefully, precisely and constantly distributed between the daughter cells during division of the material. Therefore, even before the development of experimental genetics and cytogenetics in the present century, certain research workers, for example, Boveri, Wilson, Kol'tsov, McClung, Strasburger, etc.) came to the conclusion that the chromosomes of cell nuclei should be the principal carriers of hereditary properties of the species or the form of living organisms, that is, according to the modern "cybernetic" terminology, they should be the material substrate of the controlling system of cells and the code

of hereditary information. With the development of experimental genetics the chromosomal hypothesis of heredity was proposed. It has stopped being a hypothesis for a long time, having been converted into a convincing, harmonious chromosomal theory. One of the conclusions of this theory is the recognition of an actually and objectively existing linear differentiation of chromosomes into sections corresponding to elementary hereditary factors -- genes (Bridges, 1916, 1923, 1935; Morgan, 1922, 1926, 1935; Levitskiy, 1924; Morgan, Sturtevant and Bridges, 1925; Wilson, 1925; Muller, 1926; Belar, 1928; Stern, 1931; Timofeyev-Resovskiy, 1931, 1934, 1937; Demerec, 1934; Painter, 1934; Darlington, 1937; Müller, 1937; Morgan, 1937; Kol'tsov, 1939; White, 1955). This latter standpoint was a logical conclusion based on a tremendous/^{number} of genetic, cytogenetic and cytological experiments and observations over/^a large number of species of plants and animals. At the same time, most careful cytological observations of the structure of dividing and resting nuclei (both stained and living) showed a continuous structural individuality of chromosomes during the entire life cycle of the cells, which has recently been confirmed in vivo by excellent microscopic movie films. The experimental /^{the} quantitative study of/spontaneous mutation process and that induced by various physical and chemical factors in various living organisms which have been developed during the past three decades has shown, in agreement with the main principles of the chromosomal theory of heredity, that almost all mutations are changes in the nuclear hereditary factors and that the genes possess at least ~~as~~ a final, relatively high degree of stability (for example, Muller, 1927, 1928, 1932, 1937, 1938, 1940, 1941, 1947, 1954a, 1954b;

Stadler, 1930, 1932; Stubbe, 1937, 1938; Timofeyev-Resovskiy, 1931, 1932, 1934, 1937, 1940a, 1940c). Finally, in a number of special experiments and observations the primacy of the nucleus was established in the determination of a whole series of special properties and in the general viability of the cells (for example, by Kol'tsov, 1935a, b; Astaurov, 1947, 1958; Pontecurvo, 1952, 1957; Hämmerling, 1953; Stern, 1954a, 1954b, 1956, 1958; Brachet and Chantrenne, 1956; McClintock, 1956; Mirsky, Osawa and Allfrey, 1956; Ephrussi, 1958; Lederg, 1958).

3. Therefore, even at the beginning of the 19th century the foundation had been laid for the idea that a precise distribution of special nuclear organelles between the daughter cells, --chromosomes --/which possess the capacity for reduplication before cell division underlies multiplication (this is the most typical property of living organisms).

Even at that time the importance of chromosomal material, particularly for what we now call the controlling system and the code of hereditary information, was emphasized by the establishment of the fact that in many organisms the sperm cells are very small in size (particularly by comparison with the ova), and their heads (which participate in fertilization) consists practically entirely of chromosomal material; at the same time, all hereditary features and properties of the paternal individual are transmitted through the sperm. In 1893, at a routine meeting of Russian natural scientists and physicians in Moscow a very noteworthy event occurred. The Moscow chemist A. Kolli gave a report in which he presented his calculations of the number of protein macromolecules in the smallest sperm heads (Kolli, 1893).

Chemistry of proteins and other organic

had begun
macromolecules / to be developed only at that time; therefore, the calculations of A.Kolli were very inaccurate and he, perhaps, somewhat overestimated the average number of protein molecules. However, he came to a very curious conclusion: in the small sperm heads the possible number of macromolecules was relatively small. Because the number of hereditary features and properties even of a simple organism should be quite large, the recognition of the need for associating the transmission of each hereditary feature with no more than a single molecule was the result of A. Kolli's computations; a second most important conclusion was the idea that at the basis of multiplication and heredity there should be an autocatalysis of specific molecules. The majority of biologists did not direct attention to this report at that time, and in any case they did not take a serious attitude toward apparently unnecessary calculations and chemical rationalizations. However, this report made a great impression on some of the biologists who were young at that time and among them, particularly, N.K. Kol'tsov, who began to think of the physico-chemical nature and the principal properties of the elementary biological structures.

4. N.K. Kol'tsov, at his own admission, was to a considerable degree under the influence of A. Kolli's report when he devoted a large part of his future experimental and theoretical work to the problem of the physical and chemical nature of the principal structural elements of the cell (Kol'tsov, 1936b). A number of his classic experimental investigations were devoted to the statics and dynamics of the cell. Beginning with 1916/^{his works began} to appear, devoted to the theory of the structure and multiplication of chromosomes as well as to their

controlling activity in the life of cells and ontogeny of multicellular organisms (Kol'tsov, 1928, 1929, 1932, 1933, 1934, 1935a, b, 1936a, b, 1937, 1938, 1939).

He proposed a hypothesis according to which every chromosome, or more accurately its basic constant structure (called a genoneme by Kol'tsov and corresponding to the chromoneme of cytologists) constitutes a long protein micelle (or bundles of parallel identical micelles) in which the linear molecular structures of different degrees of complexity are connected with one another by relatively simple bonds. These linearly arranged molecular structures correspond to the individual genes, according to N.K. Kol'tsov. The nucleic acids in certain other substances were considered by N.K. Kol'tsov inconstant "casings" which were absorbable by the genoneme: at that time the viewpoint was widespread according to which chromosomes did not contain any nucleic acids at all at certain stages of the cellular cycle.

N.K. Kol'tsov ascribed the capacity for autocatalysis to the micelles of the genonemes, that is, the capacity for building micelles similar to itself from the chemical material present in the cell. Such an identical reduplication of genonemes was considered by N.K. Kol'tsov the main mechanism underlying multiplication of living organisms. Therefore, in addition to the previous (associated with the development of the cellular theory) viewpoints, characterizing life -- "omnis cellula ex cellula" and "omnis nucleus ex nucleo" -- he proposed a new principle -- "omnis molecula ex molecula", having in mind the molecules or micelles of the genonemes. According to N.K. Kol'tsov, a regulated synchronous autocatalysis of the entire set of genonemes, leading to longitudinal splitting of the chromosomes during mitosis underlay multiplication. Considering

1

system
the set of chromosomes the main/controlling the development of the cell, N.K. Kol'tsov believed that the primary products by means of which the genome of the cells carries out its control of the system of specific syntheses during the ontogeny of various cells and multicellular organisms are the result of repeated but not necessarily synchronous processes of autocatalysis of the individual genes which occur during the intermitotic phases. Therefore, N.K. Kol'tsov developed a harmonious system of ideas concerning the principal phenomena associated with the chromosomal structure, their participation in cellular processes and cell division. The hypotheses of N.K. Kol'tsov were ^{not} without ^{foundation,} because they were based on the results of a whole series of precise experiments ~~1~~ and observations. The chemical models which he proposed naturally do not satisfy us to any great extent at the present time, because they were constructed on the basis of the inadequate data concerning the chemistry of nuclear structures available at that time. However, his general conception is of great interest even at the present time.

5. After the first successful experiments of G.A. Nadson and G.S. Filippov (1925) on yeasts and G. Müller on drosophila (Müller, 1927, 1928) the quantitative study of the process of mutation, both spontaneous and that produced by irradiation, proceeded at a vigorous rate (Stadler, 1930, 1932; Timofeyev-Resovskiy, 1931, 1934, 1937, 1940c; Müller, 1932, 1937, 1940, 1941, 1954a, 1954b; Stubbe, 1937, 1938). In connection with this, a new possibility appeared for studying the ^{nature of the gene.} ^{nature of the} Actually, the ^{particles and structures} which were inaccessible or difficultly accessible to direct observation could be

judged, to a certain degree, on the basis of a quantitative study of their variations. The first method used in this area was a quantitative study of the direct and reversible mutations of various genes under the influence of X-irradiation. Direct and reversible mutations of a whole series of definite genes were obtained on very extensive material and in various biological objects (Timofeyev-Resovskiy, 1929, 1930, 1932; Patterson and Muller, 1930; Johnston and Winchester, 1934; Giles, 1955), whereby in various cases either the direct or the reverse mutation predominated, and in certain cases they were of equal probability. In various cases, mutations were obtained in different directions within the limits of a series of multiple alleles (Timofeyev-Resovskiy, 1932).

The possibility of obtaining direct and reverse mutations under the influence of such a physical factor as ionizing radiation is difficult to harmonize with the idea of a complex multimolecular nature of the gene, but it is readily understandable if we consider that the ^{genes are} / physico-chemical units of various sizes (macromolecule, micelle or a more or less autonomous portion of the micelle). A further method of study of the nature of the gene was the biophysical analysis of the physical mechanisms underlying spontaneous and induced mutations. The results of such an analysis (Timofeyev-Resovskiy, Zimmer and Delbruck, 1935; Timofeyev-Resovskiy, 1935, 1939, 1940a; Timofeyev-Resovskiy and Delbruck, 1936; Timofeyev-Resovskiy and Zimmer, 1941, 1944, 1947; Zimmer and Timofeyev-Resovskiy, 1942) along with a consideration of the phenomena of direct and reverse mutations lead to the same conclusion -- that of the "monomolecular" nature of the gene. Finally, the determination of their size is still another approach to the solution

of the problem of the nature of genes. Here, two methods are possible. On the one hand, it is possible by various methods (in biological objects which have been quite well studied) to make an approximate determination of the number of genes in the chromosome and of the volume of the chromosome, on the basis of which the maximum volume of the genes may be determined approximately. By this method we come to macromolecular dimensions (Müller, 1926, 1935; Alexander and Bridges, 1928). On the other hand, the use of "statistical ultramicroscopy" with the use of the results of irradiation with fast particles having different linear ionization densities also makes it possible

to determine the approximate dimensions of the submicroscopic structures or the number and average size of the genes in the chromosome (Holweck, Zimmer and Timofeyev-Resovskiy, 1939, 1942; Timofeyev-Resovskiy, 1940a; Luria, 1940; Lea, 1946, 1947; Fano, 1941; Lea, Haines and Bretcher, 1941; Riehl, Timofeyev-Resovskiy and Zimmer, 1941; Bonet-Maury, 1942; Zimmer, 1943; Timofeyev-Resovskiy and Zimmer, 1944, 1947; Lea and Catchside, 1945; Sommermeyer, 1952). By this method ~~xx~~ macromolecular dimensions were also obtained for the genes.

N.K. Kol'tsov's idea of the "micelle-like"

6. At the present time,

structure of chromosomes may be considered more or less firmly established. Further genetic, cytogenetic, cytological, biochemical and biophysical investigations have the aim of intensifying our knowledge concerning the general nature of the structure of the chromosomes in genes. With this aim in view a study is also being made of the fine structure of chromosomes in various

stages of the cellular cycle and in various tissues, particularly the cytogenetics of giant chromosomes in the salivary glands of diptera (Belar, 1928; Painter, 1934; Bridges, 1935; Muller and Prokofjeva, 1935; Darlington, 1936; Muller, 1938, 1940, 1941, 1954a; Bauer, 1939-1942; Sax, 1941; Lea and ^{Catchside}, 1942; Thoday and Lea, 1942; Timofeyev-Rasovskiy and Zimmer, 1947; Taylor, 1953; White, 1955). The problem of the possible intragenic subdivisions and intergenic connections is being investigated by genetic and cytogenetic methods (Demerec, 1933, 1938, 1956; Mazia, 1954; Dubinin, 1935; Muller and Prokofjeva, 1935; Delbrück, 1940; Raffel and Muller, 1940; Muller, 1941, 1947, 1954a; Pontecorvo, 1952, 1957; Stadler, 1954; Green, 1955; Bonner, 1956; Levine, 1956; McClintock, 1956; Delbrück and Stent, 1957). Finally, a number of very interesting trends is being developed in the study of the biochemical and morphogenetic effects of genes. On the one hand the development of the method of injections and transplantations of ^{imago} discs in larvae of insects (Ephrussi and Beadle, 1936) has made it possible to uncover a number of specific "gene hormones" and to show general deeper interrelationships between the genetics and physiology of development (Kol'tsov, 1934, 1935b, 1938; Ephrussi and Beadle, 1936; Beadle and Ephrussi, 1937; Stern, 1954a, 1956; Hadorn, 1954; Kuhn, 1956; Mirsky, Osawa and Allfrey, 1956; Pontecorvo, 1957). On the other hand, the study of the mutation process in a number of bacteria and yeasts (first extensively developed in the works of K. Lindegren and G.A. Nadson) led to a detailed analysis of the so-called "biochemical mutations". This, in its turn, is apparently making it possible in a number of cases to approach very closely the solution of the problem of the

direct association of certain genes with certain cell enzymic processes and the nature of the primary gene product (Beadle, 1945; Demereck, 1945; Tatum and Beadle, 1945; Spiegelman, 1946; Spiegelman and Kamen, 1946; Muller, 1947; Lindegren and Lindegren, 1950; Pontecorvo, 1950, 1952, 1955; Bonner, 1951, 1956; Stern, 1956). Finally, most recently a very interesting trend has been developed on the study of genetics and biochemistry of cell populations in tissue cultures, which also apparently is making it possible to approach closely the problem of direct intracellular effects of genes (McClintock, 1956; Braun, 1958; Ephrussi, 1958; Lederberg, 1958; Puck, 1958; Stern, 1958).

7. It has been established (by means of the method of determining the absorption of various lines of the ultra-violet spectrum in chromosomes) in classic works of T. Caspersson (1936, 1950, 1956) which have already been performed that nucleoproteins are included in the composition of chromosomes. At approximately the same time, that is, in the second half of the 1930's (Stanley, 1938) pure preparations were obtained of very simple viruses, and after that it was determined that viruses and phages are also nucleoproteins.

A whole series of parallels may be drawn between genes and viruses (true, with proper precautions and limitations) (Demerec, 1933, 1938; Kostoff, 1936; Timofeyev-Resovskiy, 1940a; Lea and Smith, 1941-1942; Riehl, Rompe, Timofeyev-Resovskiy and Zimmer, 1943; Lea, 1946; Timofeyev-Resovskiy and Zimmer, 1947; Muller, 1947, 1955; Buzzati-Traverso and Cavalli, 1948; Bonner, 1956; Pontecorvo, 1957). In any case, undoubtedly the capacity (under certain conditions) of

autoreproduction

(including in the form of convariant reduplication) is common to both groups, as is also the accomplishment of certain biochemical and morphogenetic processes in the cell. Therefore, it is quite noteworthy that both groups (genes and viruses) are nucleoproteins in their chemical nature.

The virus protein, devoid of nucleic acids, loses its capacity for **autoreproduction**;

the nucleic acids specific for certain forms of phages and bacteria are apparently capable of synthesizing "their own" proteins in the cells of certain forms of bacteria and afterwards of multiplying. In connection with this, particularly considerable attention has been given recently to nucleic acids and particularly DNA (characteristic of bacteria and nuclear cell structures) and the main role has been ascribed to it in **autoreproduction** and in the

controlling activity of chromosomes (Watson and Crick, 1953a, b; Crick and Watson, 1954; Delbrück, 1954; Gamow, 1954, 1955; Rich and Watson, 1954; Gamow and Ycas, 1955; Dubinin, 1956; Crick, 1956; Vol'kenshteyn, 1958; Gamow, Rich and Ycas, 1957). The question of whether the pure species-specific DNA is adequate for the complete accomplishment of functions of control and transmission of hereditary information (characteristic of genes and chromosomes) or whether a structural union with certain proteins is needed for this (that is, the formation of species-specific nucleoproteins) is, in our opinion, still open. However, in any case it is clear that nucleic acids and their specific **reactions** with proteins in the presence, as a rule, of certain prepared nucleoprotein

"matrices" constitute a general and fundamental principle which underlies identical self-reproduction and the phenomena of heredity (and \pm of hereditary variation) in all living organisms.

8. Therefore, the problems of the nature of the elementary controlling (which accomplish **autoreproduction** and which determine the cell structures

hereditary properties and hereditary variability of living organisms) which were opposed at the end of the 19th and beginning of the present century from the general biological and cytological points of view have at the present time assumed a very specific physico-chemical form. A specific, chemical-catalytic activity and **autoreproduction** (in the form of convariant reduplication)

(Timofeyev-Resovskiy, Zimmer and Delbruck, 1935; Timofeyev-Resovskiy, 1939, 1940a) of macromolecules or micelles, which consist of nucleoproteins in the structure of which apparently the most important part is played by DNA, **underlie** life. In connection with this, the need has arisen for posing and carefully investigating the problem of the mechanisms which **underlie autoreproduction** and the specific activity of these "biological macromolecules". This has been pointed out repeatedly by biologists and physicists. However, the great complexity of the entire problem cannot be forgotten. From the large number of careful cytological observations and cytogenetic and genetic experiments it follows undoubtedly that such phenomena as the general physical mechanism of mitosis and, particularly, meiosis, homologous conjugation of chromosomes during meiosis, the crossing-over mechanism, the mechanism of formation of chromosomal reorganizations, the mechanism of mutations, the formation of primary gene products and, finally, the mechanism of reduplication of chromosomes **are** in a certain way interrelated and have a common basis in structure and in their method of **autoreproduction** of chromosomal macromolecules. Therefore, at the present time, it is hardly possible (and it is impractical to attempt) to construct a finished theory of the structure and physical mechanisms of chromosomal activity, virus activity and that of similar cytoplasmic organelles. However, it is not only possible but it is also proper to attempt to make an analysis of the

specific physical mechanisms associated with the problem of a structure and activity of the principal biological controlling and convariantly-reduplicating structures. The main task of such investigations is primarily the problem of the possibility of constructing, on the basis of certain /physical parameters, such models of specific mechanisms which would satisfy the requirements made on these models on the basis of empirical observations (chiefly with respect to volume, time intervals, sources of energy, the nature of the chemical medium and the forces participating in the processes). The construction of models of this kind certainly requires the very close cooperation of physicists, chemists and biologists; they should mutually acquaint one another with their respective special methods and material to such degree as to work out a common language and adequate mutual understanding.

9. A small group of physicists and biologists have made an attempt at/combined solution of certain of the principal problems of the theoretical biophysics of phenomena associated with ~~autoreproduction~~ of biological macromolecules. In subsequent publications of this series a brief description will be given of the results of the theoretical analysis (and subsequently, partly also of the experimental analysis) of such specific problems the solution of which is absolutely necessary to us for a future construction of a theory of ~~autoreproduction~~ and specific actions of elementary biological structures.

10. Finally, one other comment should be made. This year we are celebrating a double Darwinian anniversary: 150 years since the date of birth of Charles Darwin and 100 years since the time of publication of his "Origin of Species". Charles Darwin created a natural historical theory of evolution of

living organisms on our planet, discovering the principle of natural selection. Just as the principle of universal gravitation and the laws of mechanics formulated by Newton created the possibility of constructing a harmonious physical picture of the world so the theory of evolution and the principle of natural selection formulated by Darwin created the possibility of constructing a strictly scientific picture of living nature. Modern "microphysics" is deepening and expanding the physical picture of the world, not repudiating but rather altering and perfecting the macrophysical ideas created by Newton. In a similar way, the Darwinian theory of evolution is being made more precise and being deepened by modern cytological, genetic, physiological, biogeocoenological, biochemical and biophysical ideas unknown to Darwin. The fact that the principle of natural selection is inevitably associated with convariant reduplication of macromolecules and stems directly from it is particularly worthy of attention. Therefore, the discovery of the mechanisms of autoreproduction represents of elementary biological structures/ a considerable deepening of our ideas concerning the mechanisms and routes of evolution and the further discovery of the significance of selection as a factor creating controlling, "cybernetic" systems (Timofayev-Resovski and Rompe, 1959) for living organisms (and only for them and their activity).

11. It is essential here briefly to detail the terminology of the phenomena of autoreproduction. We use the term "self-reproduction" or "identical self-reproduction" in its application to individuals of various forms of living organisms. The term "autoreproduction" in a general form is applied to the

phenomena of autocatalysis of biological macromolecules and elementary cytological structures. Finally, the term "convariant reduplication" characterizes multiplication by means of duplication (for example, longitudinal splitting of chromosomes before mitosis) of elementary biological structures containing a code of hereditary information; "convariant" designates the fact that in the event of occurrence of mutations the appropriate cytological structure or biological elementary unit (chromosome, plastid, mitochondria, virus particle or phage) reduplicates itself in a new altered form.

Resume

The history of the gradual formation of modern concepts of the physico-chemical nature of elementary cell structures underlying multiplication, the control of ontogeny and the phenomena of heredity and hereditary variation since the end of the 19th century is presented in brief.

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The Mechanisms of Autoreproduction of
Elementary Cell Structures

**II. Physical Basis for the Spiral Shape of
Certain Macromolecules and the Possible
Mechanism of Autoreproduction of Deoxy-
ribonucleic Acid.**

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1. As a result of the progress which has been made in recent years in the
study of the structure of polymers it has been found that macromolecules of
linear polymers which are constructed of several chains have, as a rule, a
spiral shape. It is specifically these polymers which constitute the basis of
the elementary biological structures. It is particularly interesting that the
shape of twin spirals is shown also by molecules of desoxyribonucleic acid
(DNA), which plays such an important part in the life ^{of} the cell; the structure
of the DNA molecules themselves indicate the possibility of their autoreproduction.
According to the modern views the basic features of the structure of the DNA
molecule amount to the following (Watson and Crick, 1953a, 1953b; Crick and
Watson, 1954). Each of the two chains of DNA consist of a succession of nucleo-
tides, which in their turn consist of a phosphoric acid residue, pentose
(desoxyribose) and a nitrogenous base. The nucleotides are different from one
another in the type of base. DNA includes four nitrogen bases: adenine,
guanine, thymine and cytosine. The chains, as has already been mentioned, are
coiled in the form of twin spirals and are connected with each other through
the nitrogen bases by hydrogen bonds. It has been shown that in the DNA
molecule the adenine can form such a connection only with thymine; guanine, only

with cytosine. Therefore, although the succession of nucleotides in the two DNA chains is different, their order in ^{one} chain determines the same positions in the other chain. If we imagine the chains were divided and that a new chain began to be built near each of them, as a result two twin spirals would be created which are identical with the original. However, such an explanation of the autoreproduction process of DNA runs into certain difficulties: it is not clear what the mechanism of separation is of the chains which are twisted in a spiral shape. A number of works has been devoted to attempting to explain this problem but at the present time we can not consider it solved as yet (Bloch, 1955; Gamow, 1955; Platt, 1955).

Works on the study of DNA have led to an interpretation of its structure and have made it possible to create a model of the DNA macromolecule in the form of twin spirals. However, to date no satisfactory attempt has been made to find an explanation for the advantage specifically of this form of DNA macromolecule from an energy point of view. The simplest explanation, at first glance, which connects the stability of the DNA macromolecule shape with its correlation with the condition of least deviations from the optimum valence angles and interatomic distances is inadequate, because the structure which we observed is not the only one which would satisfy this condition. Therefore, we have analyzed the problem of the stability of such structures from an energy point of view. A strict mathematical treatment of this question is given in another place (Plishkin, Luchnik and Taluts, 1959); here, we should like to present a qualitative analysis and ^{draw} the main conclusions, discussing some of the biological consequences which stem from them.

2. Let us consider two chains of particles reacting both with the particles of the same and with the particles of the other chain. The forces acting in the molecular structures possess the property such that at short

distance the interacting particles are repelled, and at great distances they are attracted; therefore, the potential energy of the reaction of two particles depending on the distance between them changes in a manner such as has been depicted in Fig. 1, that is, it has a single minimum which increases indefinitely when the particles are brought together and has a tendency to go down to zero when they are separated. We should like to call to mind the fact that the state corresponding to the minimum potential energy is the stable one.

Let us imagine two parallel chains of particles reacting with each other (Fig. 2a). Within one and the same chain the energy of the reaction lessens quickly with the number of the particles; practically, it may be considered that each particle reacts only with the two neighboring particles of the same chain. As far as the reaction with particles of the other chain is concerned, the distances and, therefore, the energy of attraction between ^acertain particle of one chain and several adjacent particles of the other chain are quite similar. From this, it follows that a well-defined and ^{equal} distances between the chain particles correspond to the stable state. Therefore, a strictly parallel arrangement of the chains is unstable and, therefore, if the chains are arranged in parallel the particles would be displaced in such a way that the distances BA', BB', BC', etc. would be equal (Fig. 2b). Here, the particles would be arranged at the apices of tetrahedra of the ABB'C' type in Fig. 2b. Therefore, the stable configuration should correspond to a number of tetrahedra in contact with one another. With the reaction of each particle of one chain with four particles of the other tetrahedron, they should come in contact at the borders; thereby, a configuration arises in which the particles of the two chains are arranged like the coils of twin spirals (Fig. 3). Analysis shows that this configuration is the only stable one for the model under analysis.

Therefore, for a system consisting of two reacting chains of particles the spiral shape is an inevitable consequence of the fulfillment of the elementary stability conditions of the structure.

We should like to note that with the reaction of each particle of one chain with less than three particles of the other no stable configuration is obtained. With a reaction with three particles (which corresponds to contact of the tetrahedra along the edges) a pair of spirals is obtained with a certain arbitrary orientation. With four or more reactions a rigid twin-spiral structure is obtained. A similar analysis is obtained from equilibrium conditions of a spiral configuration of a system consisting of three chains. What has been stated remains justified also for chains consisting of particles of different kinds. Here, a twin-spiral structure is also possible in which transverse links do not intersect the axis of the spiral, which resembles even more the DNA model proposed by Watson and Crick.

3. What can these conclusions give us for the purpose of learning about the ~~autoreproduction~~ process? At the beginning of the article mention was made of the problem of separation of the strands of the twin DNA spirals, which can be considered the premise for ~~autoreproduction~~. The main conclusion of the present work amounts to the fact that the twin spiral structures correspond to the only minimum potential energy system. Hence, it follows that separation of the strands is practically impossible, because such a separation corresponds to an increase in the potential energy and requires the assumption of the existence of a prolonged effect of controlling forces (torques) on the DNA molecule. Therefore, it is not very probable that separation of the strands ~~precedes autoreproduction~~. It is something different again if we imagine that the separation occurs during or after ~~autoreproduction~~, because the equilibrium conditions for a system which arises thereby will naturally be different. We assume that the separation of the chains occurs

during reduplication, because otherwise it would be hard to imagine how the conversion can occur of a quaternary spiral into two twin spirals.

In this case the following mechanism of ~~autoreproduction~~ can be suggested, which, naturally, is not the only possible one. Low molecular-weight precursors are attached to the DNA molecule, but the longitudinal links between them are not formed until the "matrix" (mother molecule) collects all the material for the purpose of building the daughter molecules. The separation of such a quaternary structure is entirely possible, because not all the longitudinal associations in the DNA molecule are covalent, as was shown in the work of Dekker and Schachman, 1954. In accordance with the DNA model which they proposed it consists of minimum chemical units of a relatively low molecular weight connected with quite weak electronic bonds, whereby the sites of these "dynamic breaks" are at different points in the two chains, which produces stability of the molecule. Therefore, with the opening of the dynamic breaks the splitting into two DNA molecules can occur entirely in a manner such as has been schematically represented in Fig. 4. A reduction in the ion concentration in the surrounding medium, which, as we believe, should occur after the attachment of the precursors to the maternal chromosome, can contribute to this opening. It is interesting to note that in the work of P. S. Zyryanov (1959) the idea is presented that a reduction in the ion concentration should lead to a cessation of the effect of homologous attraction forces between the chromosomes. These considerations speak for the hypothesis suggested by Anderson (1956), according to which the events which accompany cell division are related to changes in the equilibrium of the polyelectrolytes. Certain authors (for example, Levinthal and Crane, 1956) have analyzed models in which an untwining of the old spiral occurs simultaneously with

autoreproduction. .. From an energy point of view, this is also quite possible. However, we give preference to the mechanism which has been suggested (assembly of daughter strands with subsequent separation through dynamic breaks) based on the following considerations. Untwining requires a successive assembly and combination from the end of the molecule (principle of the "lightning" zipper). Thereby, the process of assembly, to be sure, should be considerably slower than simultaneous assembly throughout the length of the DNA chain. In addition, in this case the existence of a special controlling mechanism is necessary which would not permit the onset of **autoreproduction** in the absence of an adequate quantity of "raw material". Otherwise, situations would occur which might be lethal for the cell. Preliminary assembly without longitudinal combination may be precisely such a controlling mechanism. It is interesting that certain experimental data indicate that **autoreproduction** of chromosomes can actually occur a considerable time after the doubling of the DNA content in the cell (Schwartz, 1954; Tsarapkin, 1959).

It should be emphasized, however, that the conclusions drawn are justifiable for an analysis of the model and only tentatively justified for actual DNA molecules. Here, attention should be directed to the fact that in our model each particle of/^{one} chain reacts with several particles of the other chain, because the Watson and Crick model considers only the paired reactions between the opposite nucleotides. However, data concerning the migration of energy along the DNA molecule for very great distances (Steele and Szent-Györgyi, 1957) speak for the existence of reactions (probably of the resonance type) between purine and pyrimidine bases along the axis of the molecule. Proof of the existence of such reactions would eliminate this difference between our model and the real DNA. In addition, the surrounding medium should exert an influence on the behavior of the actual molecules; this was not taken into consideration in

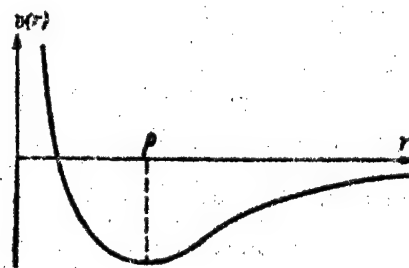


Fig. 1. Relationship of potential energy of reaction between two particles $v(r)$ to the distance between them, r .

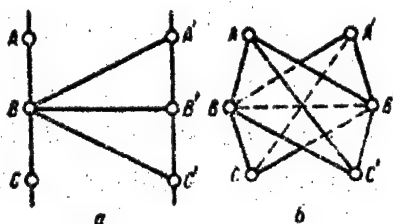


Fig. 2. Original position of particles in model under consideration and reaction of particle B with the other particles (a); arrangement of particles corresponding to minimum potential energy of the reaction (b).

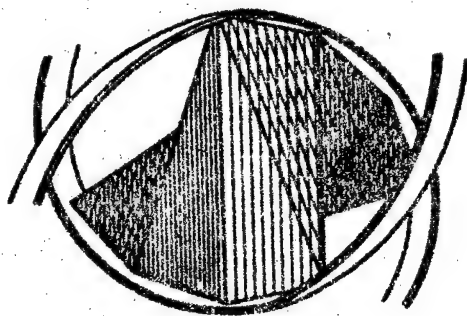


Fig. 3. Configuration of pair of chains corresponding to minimum potential energy of the reaction.

If the particles are arranged at the apices of the tetrahedra which are in contact with each other at the borders, the chains are converted into coils of twin spirals.

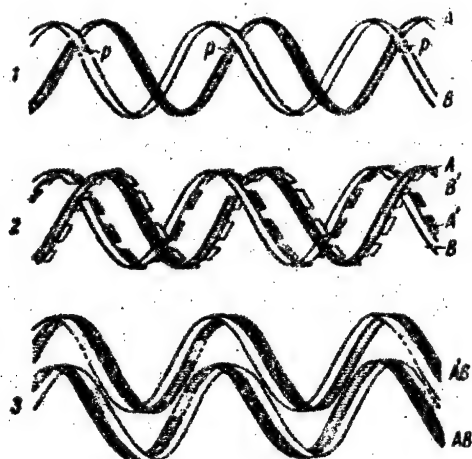


Fig. 4. Scheme of autoreproduction and separation of the DNA molecule. A and B) complementary strands of the original molecule; A' and B') newly constructed strands; p) places of dynamic breaks.
1. Original molecule; 2. new nucleotides are attached to the original filaments, but no longitudinal bonds have been formed, thanks to which separation of the molecules is possible; 3. two DNA molecules after separation and the formation of longitudinal bonds following it.

the present work either, the subject of which is an analysis of the inner forces of the system.

Conclusions

1. The stability conditions of systems consisting of two molecular chains have been analyzed, and it has been shown that under conditions of the model being analyzed the system has the only minimum potential energy corresponding to the twin-spirals configuration.

2. A hypothesis has been proposed according to which the attachment of low molecular weight precursors to the original molecule occurs before separation of the strands of this molecule during the course of ~~antireproduction~~ ^{antireproduction} of DNA, and the formation of longitudinal bonds is accomplished after separation.

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[See 4 figures on next pages].

Mechanisms of Autoreproduction of Elementary
Cell Structures

III. The Nature of the Attraction Forces Between
Chromosomes.

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1. At a certain Stage of maturation of sex cells during meiosis an interesting phenomenon is observed of attraction of the homologous portions of two homologous chromosomes of each pair (the so-called "conjugation"). The experimental facts indicate that the attraction is most intense between the homologous portions of these chromosomes. Actually, if a "reorganization" of the inversion type occurs in one of the two homologous chromosomes, for example, under the influence of ionizing radiation the intensity of the attraction forces in the area where the inversion occurred weakens substantially, because the areas opposite to one another are not homologous. From this it follows that any attempt to understand the nature of these forces should be based on an analysis of the chromosomes as spatial structures.

2. As far as we know, the first attempt at explaining the forces of homologous attraction were made by Jordan (1938), who considered the chromosome a punctate harmonic oscillator. If Jordan's results, which stem from too much simplified a model of a chromosome, can be transferred, to any degree, to actual chromosomes, it should be done with great caution. Friedrich-Freksa (1940) analyzed a spatial model of a chromosome and considered it to be a chain with diffuse fixed electrical dipoles.

It seems to us as though there are no convincing arguments on behalf of such a model and its stability. Recently, Yos, Bade, and Jehle (1957) analyzed

a more complex macromolecular model (which a chromosome is essentially),
representing a system of ^{harmonic} oscillators with a certain frequency spectrum
concentrated at a point -- the center of the spherical macromolecule.

This punctate model of a macromolecule is convenient and justified for a description
of the attraction forces between the macromolecules located at a distance exceeding
their linear dimensions. Such a model, as these authors have shown, leads to
interesting qualitative conclusions concerning the nature of the attraction forces
between the macromolecules. The author of the present article analyzed (1959)
a more general model of a chromosome, free of defects inherent in the models
described above, and gave a consistent mathematical treatment of this model on
the basis of the method of collective reactions. The aim of this article
consists in giving a more readily available qualitative presentation of the
principal results of the work mentioned.

3. Let us consider a chromosome as an electrically neutral chain
consisting of structural groups of atoms of various kinds. The chromosomes
which are not homologous differ from one another in the number of structural
groups, the order of their arrangement in the chromosome and ⁱⁿ differences in
the structure of these structural groups. Since the chains as well as their
constituent parts -- the structural groups of the atoms -- consist of a large
number of electrons and atomic nuclei statistical rules and regulations are
applicable to such systems. Specifically, fluctuations occur in such systems in
the density of the atomic nuclei and in the electrons in the vicinity of their
average values. This picture of fluctuations may be represented in the following
way. Let us separate out an imaginary small area with fixed borders within a
structural group of atoms, and we ^{shall} observe ^a number of atomic nuclei and
electrons within this area at various times. It turns out that the instantaneous

number of atomic nuclei and electrons will be different at different times, that is, the number of atomic nuclei and electrons will undergo fluctuations (variations) in the vicinity of a certain average value. Since the number of electrons and atomic nuclei per unit volume undergoes fluctuations the density of the electrical charge will undergo fluctuations also, and the density of the electrical charge, changing in time, creates an electromagnetic field in space. Therefore, the two chains which are electrically neutral as a whole can interact through electromagnetic fields excited by fluctuations in the density of the electrical charge. These fluctuations can be characterized by their spectral composition. Let us explain this by an example. Let us assume that at a certain point in space there are two closely arranged charges of opposite signs, and the distance between them varies at a frequency ω_1 ; such a system is called "a harmonic oscillator". If at this point in space there be placed two other similar charges which, however, vary with respect to each other with a frequency ω_2 and if this process be continued, then at this point in space at which all the oscillators are located the density of the electrical charge will vary with a ^{range} of frequencies: of $\omega_1, \omega_2, \dots$ this range of frequencies is also a spectral characterization of the ρ /fluctuations in the density of charge. We should like to note that hereby we are not taking into consideration the reactions between the oscillators. Consideration of these reactions leads to a change in frequencies. In the event of a study made of the fluctuations in the density of the charge in the chains we can imagine these fluctuations as equivalent sets of harmonic oscillators, whereby the frequency spectrum of these oscillators is determined by the law of reaction between the particles in the chain and ^{the} ^{the} distribution of average particle density along the chain.

4. At the present time, quite effective methods ~~is~~ exist for computing the frequency spectrum. Such calculations show that the frequency spectrum of a system consisting of heavy (atomic nuclei) and light (electron) particles has two branches. One branch corresponds to the slow variations of the heavy particles (low-frequency oscillations), and the other, to the fast oscillations of the light particles (electrons). It is not hard to explain this situation by qualitative considerations. Since the frequency of the oscillations ω is proportional to the reaction of the particles and inversely proportional to the mass it follows from this that the frequency of the oscillations corresponding to the movement of heavy particles will be small, because their masses are great, while the electrical charge determining the intensity of the reaction has the same order of magnitude for both electrons and atomic nuclei. For the purpose of graphic clarity of the relationship of the oscillations to the mass an example may be presented with a weight suspended ^{from} a spring (Fig.1). The greater the weight at a given spring elasticity the lesser the frequency of its oscillations. The low frequency oscillations describing the movement of atomic nuclei make an insignificant contribution to the intensity of the electromagnetic field of fluctuations by comparison with the high-frequency ~~in~~ oscillations. This occurs because the light particles (electrons) follow the movement of the heavy particles practically instantaneously and compensate ^{for} the volumetric positive ^{the} charge produced by fluctuation in the density of the numbers of atomic nuclei. The high-frequency oscillations corresponding to the movement of the light particles lead to the formation of the greatest volume charges. As a matter of fact, if fluctuations in the electron density occurred at any point in the chain the atomic nuclei, which possess a great mass, would not undergo any notable

displacement during the existence of this fluctuation and would not compensate for the negative electrical charge of the electron fluctuation. This conclusion indicates that the electromagnetic fluctuation field is created chiefly by high-frequency oscillations.

5. Based on the average density of the number of electrons in the chain the order of magnitude of the frequency of electronic oscillations can be estimated. These oscillations cannot be excited by thermal movement of the particles, because the energy of thermal movement of the particles KT is inadequate to excite a quantum of vibration having an energy of $h\omega$ (K is the Boltzmann constant; T --the absolute temperature; h --the Planck constant; ω --the minimum frequency of the high frequency oscillations). However, according to the laws of quantum physics even at a temperature of $T=0$ there is a final value of the amplitude of oscillations, the so-called "amplitude of zero oscillation". Therefore, the electromagnetic field of high-frequency fluctuations has a quantum nature and in practice does not depend on the temperature, since the amplitude of zero oscillations does not depend on it.

6. Let us examine further the matter of the reaction of two parallel chains by means of electrical fluctuation fields. A detailed mathematical analysis of the problem shows that attraction forces will occur between such chains, and the magnitude of these forces will be proportional to the degree of overlapping of the spectra of the oscillation frequencies of the electromagnetic fields of these chains. For identical (homologous) chains the spectra overlap completely or simply coincide if the chains are arranged so that identical structural groups of atoms are opposite one another (Fig. 2). Therefore, the intensity of the

attraction will be greatest specifically in this case. In other words, the attraction of chains is of a resonance nature in the sense that when the oscillation frequencies of the electromagnetic fluctuation fields of the chains coincide the attraction is maximal. The qualitative picture of the reaction phenomenon of the chains can be clarified through the example of a simple mechanical system. Let us analyze the example of the oscillations of two connected weights (Fig.3). In case a (Fig.3) the excitation of the oscillations of the left-hand weight (m) causes oscillations in the right-hand weight (m), whereby the maximum amplitude of oscillation of the right-hand weight reaches the maximum amplitude of oscillation of the left-hand weight. In case b (Fig.3) the weights are different, and the maximum amplitude of oscillations of the right-hand weight will be considerably less than the maximum amplitude of the left-hand weight. From this example it is seen that the effect of the left-hand system on the right-hand system will be maximal when these systems are identical, that is, when they are in resonance. A similar picture is observed in the ^{case} of two electrical oscillators. If the oscillators are identical excitation of one of them will produce resonance oscillations in the other. In studying the problem of the reaction of two chains the problem essentially amounts to an analysis of the reaction of a large number of oscillators of one chain (representing fluctuations in the density of the electrical charge) with the oscillators of the other chain. It is not hard to see that here the maximum reaction ^{between} the chains will occur in the event they have the same oscillation spectrum, that is, are in resonance or, in other words, are identical (homologous) and homologous structural groups of atoms are superposed,

as is shown in Fig. 2.

7. Therefore, the attraction forces between the homologous chromosomes are produced by the reaction of \pm electromagnetic fields created by the zero fluctuations of the density of electrical charge in the chromosomes, which may be considered neutral macromolecules extended as a whole. These forces are similar to the van der Waals forces in their nature.

8. Let us analyze further the influence of the surrounding medium on the reaction between the chromosomes. The surrounding medium in which the chromosomes are immersed contains aqueous solutions of salts which are electrolytes. Between the chromosome, which is a giant macromolecular structure, and the electrolyte it is natural to suppose the existence of a contact potential difference creating a polarized cloud of ions (Fig.4). When such structures are brought together the resonance forces of attraction are equalized by the coulomb forces of repulsion between the polarization clouds. The competition of these forces can, under certain conditions, lead to the formation of stable bonds between two identical chromosomes.

The magnitude of the energy which must be used for removing particles from one another to quite a great distance, at which they practically do not attract ^{each} another, is called "the binding energy". The magnitude of the binding energy ($E_{be.}$) depends on the concentration of ions in the medium. It may be supposed that the dimensions of the ion cloud surrounding the chromosome will be greater, under certain conditions, the lower the concentration of ions. Here, the coulomb forces of repulsion of the ionic clouds of two chromosomes will, at

low concentrations, interact at comparatively great distances, where the resonance fluctuation forces are still small. Then the binding energy of the two chromosomes will decrease with a reduction in the ion concentration (Fig.5,c). If the binding energy is no greater than the energy of the thermal chaotic movement of the molecules of the medium in/ which the chromosomes are immersed in its magnitude the bound states of the two chromosomes can be destroyed by thermal movement.

9. We came to the conclusion that at this temperature the answer to the question of whether bound states of the two chromosomes will exist at this temperature or not depends on the concentration of ions. Therefore, in the model under analysis the homologous chromosomes can form bound states which break up at a constant temperature with a reduction of ion the/concentration of the medium.

One of the causes leading to a reduction in the ion concentration of the medium may possibly be the fact of duplication of a set of chromosomes during the process of cell division.

10. A detailed mathematical analysis of the relationship of the binding energy to the distance between the chromosomes leads to an interesting result. It is shown that under the influence of considerable fluctuation in the thermal movement of the molecules of the medium a local approximation of the interacting chromosomes may occur. If the forces of chemical attraction operate here an exchange of homologous chromosome sections can occur. Possibly this phenomenon underlies crossing-over (normal exchange of sections of homologous chromosomes during conjugation in meiosis). However, this matter requires further special investigation.

11. In conclusion, we should like to formulate briefly the principal results.

1) The attraction forces between chromosomes, which may be considered macromolecules, are brought about by electromagnetic fields the sources of which are quantum fluctuations in the density of the electrical charge.

2) The potential energy of attraction between chromosomes (the binding energy) has its maximum absolute value (with consideration of the sign this energy is minimal, because the attraction forces have a negative sign) for homologous chromosomes which have homologous sections superposed on them. The potential energy of attraction coincides with the free energy of the system in the event of isothermal processes. In actual systems conditions are always realized with a minimum free energy, that is, the homologous chromosomes will come together.

3) The dissolution of the bound states of two homologous chromosomes can occur when there is a reduction in the concentration of the ions in the medium.

4) In the model under analysis a local approximation of chromosomes may occur under the influence of a considerable fluctuation in the thermal movement of the molecules in the medium; this approximation possibly underlies the crossing-over phenomenon.

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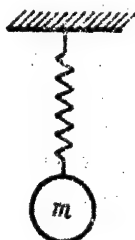


Fig. 1. Very simple mechanical model illustrating the relationship of the oscillation frequency to the mass of a body (m).

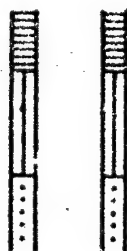


Fig. 2. Homologous structural groups of atoms "superposed" on homologous chromosomes.

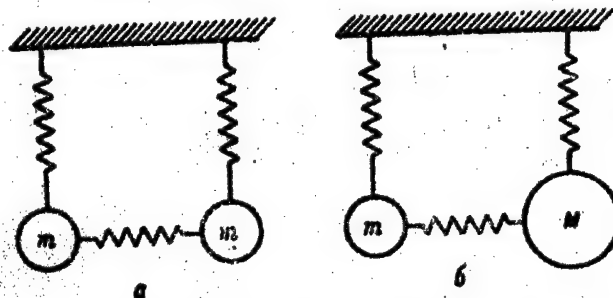


Fig. 3. Resonance fluctuations of two weights in case a. In case b, there is no resonance.

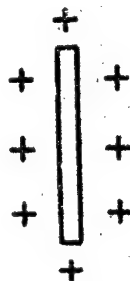


Fig. 4. Macromolecules surrounded by polarization cloud of ions.

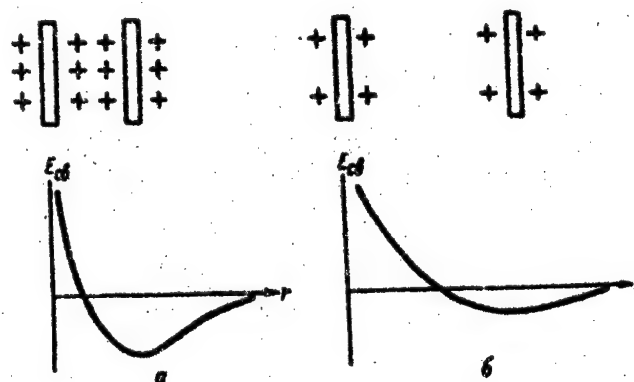


Fig. 5. The relationship of the binding energy of two chromosomes to the ion concentration. In case a, the concentration of ions is greater than in case b.

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The Mechanisms of Autoreproduction of Elementary Cell Structures

IV. A Single Possible Mechanism of Reduplication of Chain Molecules

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1. Whereas current information concerning the structure of the DNA molecules, based on reliable data of chemical and roentgen structural analysis, is quite complete, the mechanism of reduplication of these molecules remains very unclear. It may be asserted only that with an approach to the analysis of the mechanism of reduplication two facts have to be taken into consideration: first, the elementary processes which occur during reduplication should be permissible from the viewpoint of the geometric structure of the DNA molecules and, secondly, the entire process of reduplication should occupy no more time than the time of the interphase. Further, it may be supposed that the new DNA chain is synthesized from individual nucleotides, which are present in the nucleus at the beginning of reduplication. Taking into consideration the first circumstance it must be supposed that autocatalytic reproduction of the twin DNA spiral with accurate reproduction of the sequence of nitrogen bases arranged within the spiral and screened from the surrounding medium by pentose-phosphate groups is possible only when these bases can react with the nucleotides of the environment with their own chemically active groups. For this purpose a break in the hydrogen bonds within the spiral is necessary. Therefore, it must be supposed that the reduplication process includes three stages: 1) reorganization of the twin DNA spiral with a

transition to a configuration suitable for the construction of a second similar twin chain; 2) construction of the second ^{double} chain by means of attachment to the original nucleotides from the environment; 3) separation of a new chain from the old one and a transition of both chains to a normal configuration. These three stages may occur either in sequence or, in part, simultaneously. In the former case the new stage does not occur as long as the whole chain has not gone through the preceding stage. In the second case the shift of stages in various parts of the chain does not occur simultaneously, so that at any given moment the new chain is proceeding only in individual sections along the axis of the old macromolecule.

2. The possible mechanisms of the first and third stages have been discussed in the works of Plishkin, Luchnik and Taluts (1959) and Luchnik and Taluts (1959). There is still no information concerning the mechanism of the second stage, and specifically it is unknown whether specific forces characteristic of such a complex physico-chemical systems as the cell nucleus act during this process or whether autoreproduction of the chain DNA molecules proceeds by means of a simple physical mechanism. The experimental solution of this problem meets with great difficulty. Therefore, its theoretical analysis is of interest. By means of comparing the results of calculations with certain experimental data the correctness of the model selected may be checked and an approach made to elucidating the nature of the reduplication of the mechanism. In the work of the authors (1959) an attempt was made at such a theoretical investigation of the second stage of the reduplication process in the supposition that this stage

proceeds simultaneously along the entire length of the chain. Then, it may be analyzed without regard for the mechanism of the first and the third stages. In the present communication the main suppositions, ideas of the computation and the results of this work are being presented.

3. The following simple model of a physical process was made the basis of the calculation of the reduplication kinetics in our work (1959). Let us suppose that the nucleus represents a spherical drop of a homogenous fluid with a viscosity η and occupies the volume V . In it the molecular chains constructed of particles (nucleotides) of four kinds are arranged haphazardly, as are also the freely diffusing particles from which new chains ~~are to be~~ constructed; here, the number of free particles is somewhat greater than the number of corresponding particles in the chains, so that there is a certain excess of "building material". The chains are in the second stage of reduplication process, that is, the hydrogen bonds are broken in them and every particle of the chain may attach a free particle from the surrounding medium to itself. In other words, the field of force in the vicinity of the chains is such that they form potential pockets in which the free particles may enter. Evidently, the depth of the potential pocket, which determines the attraction forces of the particles, depends both on the kind of particle in the chain forming the pocket and on the kind of particle entering it (Fig.1). Let us designate the depth of the potential pocket, which is measurable, for example, in electron-volts (1 ev=23 kcal/mole) by $\mu_j^{(i)}$, where j designates the kind of pocket; i , the kind of particle entering it. The particle entering the pocket can also leave it if it acquires sufficient energy through

the fluctuation of/energy of thermal oscillations. The probability of leaving the pocket is equal to

$$V_i^{(j)} \exp - \frac{U_i^{(j)}}{kT}, \quad (1)$$

where $V_i^{(j)}$ is the frequency of oscillation; k , the Boltzmann constant; T , the absolute temperature. It is assumed that the particles entering the neighboring potential pockets do not interact. The mechanism of circulation of the neighboring particles not attached to the chain is considered to be diffusion of the molecules in the fluid, where the particle is at a certain place (more accurately, accomplishes its thermal oscillations in the vicinity of a certain temporary equilibrium state) for a short time, and then moves along the length

in an arbitrary direction for an intermolecular distance of the order of δ . During these circulations the particles collide with molecular chains and enter the potential pockets.

4. The number of particles entering the potential pockets per unit time can be calculated. It is equal to aNC , where $a = \frac{1}{2} R^2 \frac{v}{V}$, where R is the radius of the particles (it determines the width of the pocket), N is the number of potential pockets, C is the number of particles in the fluid at the original moment, v is the average rate of migration of the particles in the fluid. It is determined by the viscosity of the fluid η and $v = \frac{kT}{2\pi R \eta \delta}$. The coefficient a characterizes the frequency of collisions of the diffusing particles with the chains. After a single particle has entered a potential pocket the number of free pockets becomes equal to $N - 1$, and in the fluid C becomes one particle. Therefore, at the time when n potential pockets are occupied in the chain the number of empty

pockets will be $N-n$, and the number of free particles, $C-n$. Therefore, the increase in the number of n particles in the time dt which are resting in the potential pockets will be equal to

$$dn_1 = \pi R^2 \frac{v}{2V} (N-n) (C-n) dt. \quad (2)$$

The decrease in n during this time was brought about by the particles' leaving ("evaporation") the potential pockets because of energy fluctuation. The probability of evaporation of a single particle per unit time is determined by formula (1), and if at the time t there is a total of $n(t)$ captured particles the complete number of particles evaporated in the very short interval of time dt is equal to

$$dn_2 = n V \exp\left(-\frac{U}{kT}\right) dt \quad (3)$$

A complete change in the number of particles n during the time dt is equal to the difference dn equals $dn_1 - dn_2$. Substituting the values (2) and (3) here and dividing by dt , we obtain the differential equation

$$\frac{dn}{dt} = \pi R^2 \frac{v}{2V} (N-n) (C-n) - n V \exp\left(-\frac{U}{kT}\right). \quad (4)$$

Solving this equation we find the number of particles which are in the potential pockets at the time t . The solution should satisfy the following initial condition: the number of particles which are in the potential pockets at the beginning of the process should be equal to 0 , that is, $n(0)$ equals 0 . The construction of the new chain will be completed at the time $t = t^*$, where the number of captured particles or, which is the same thing, the number of pockets filled is equal to the total number of pockets, that is, $n(t^*)$ equals N .

However, the possibility has not been excluded that with certain values of the constants included in the equation (4), $n(t)$ never reaches the value N . This should be expected in a case where the attraction forces between the engulfed particles and the chain are so weak (the potential pockets are superficial) and the temperature is so high that the particles do not stay in the potential pockets for any length of time, and in the course of time a dynamic equilibrium is established between the captured and the free particles.

5. The equation (4) describes the relationship $n(t)$ only for the specific case where there are particles of one kind and pockets of one kind. In order to describe a process of duplication of polynucleotide chains constructed of particles of four kinds it is necessary to formulate an equation of type (4) for the particles and pockets of four kinds (a system of so-called "kinetic equations"). A system of 16 equations with 16 unknown functions is obtained. The solution of such a system presents considerable mathematical difficulties. Nevertheless, it may be expected that the principal characteristics of the kinetics of the construction process of the chains will become manifest in the examination of the construction of chain from particles of two kinds. In this case, the system contains four equations of type (4). However, with such a simplified formulation it is not possible to solve the problem in a general form, strictly speaking. In such cases the method of successive approximations may be used, which amounts, in first, to finding the solution $n(t)$ of the simplified equation (or system of equations), substituting this so-called zero approximation into the right-hand portion of the exact equation, and solving

the new equation obtained in this way. In our case, we are introducing the following simplifications into the zero approximation equation. For particles of both kinds we shall consider that the coefficients of collision a are the same, the number of particles (or pockets) in the chains are $N_1 = N_2 = N$; the original number of particles in the fluid, $C_1 = C_2 = C$; the frequency of thermal oscillations of the particles $V_1 = V_2 = V$; the energy of the particles in "their own" (the deepest) pockets is $U_1^{(1)} = U_2^{(2)}$ and the energy of the particles in the other pockets $U_1^{(2)} = U_2^{(1)}$, but $|U_1^{(1)}| \neq |U_1^{(2)}|$. Naturally, at the beginning of the process the particles will move about in a chance manner along the pockets of both kinds, but afterwards the less firmly bound particles will evaporate more rapidly, and in the course of time a configuration will be established in which all the particles occupy the deepest pockets. The order of arrangement of the particles of various kinds in the new chain will correspond in the same way to the order of the arrangement in the old chain if the potential pockets for particles entering "their own" place are deeper than for the particles in the other places, that is, if $|U_1^{(1)}| > |U_1^{(2)}|$. Then, the probability of leaving "their own" pockets will be considerably less than that of leaving the other pockets.

6. The assumptions which have been listed make it possible to simplify and solve the system of kinetic equations in the zero and first approximations and to obtain, as a zero and more accurate first approximation, the relationship of the number of pockets of kinds 1 and 2 to the time t which are occupied by their own and by foreign particles (Fig.2). Even through the examination of the

zero approximation it becomes clear that not all the parameters of the problem can be given arbitrary values. It turns out that in order for it to be possible that the construction of the chain be accomplished in a certain finite time the following condition ^{should} be fulfilled.

$$\frac{\text{Frequency of break-outs from their own pockets}}{\text{Frequency of collisions}} < \frac{\text{Excess of particles in the fluid}}{\text{Number of pockets,}} \quad (5)$$

that is, the particles should remain quite firmly in their own pockets. In order to estimate numerically the time t^* for the reduplication of the chain it is necessary to give the values of the parameters included in the system of kinetic equations. Unfortunately, many of them, for example the viscosity of the fluid, are known with a very low degree of accuracy, and with reference to the binding energies

$U_2^{(1)}$ and $U_1^{(2)}$ it can only be supposed that they are of the order of 1 ev.

Since there are not so many of such parameters ($U_1^{(2)}$ is not included) in the solution of the zero approximation, let us first estimate the time t^* in this approximation (equation 7) of our work (1959), using the following values for our parameters. The temperature $T = 300^\circ \text{K}$ (room temperature); the viscosity of the fluid in which the nucleotides are diffusing is of the order of the viscosity of water, $\eta = 0.01$ gram/centimeter second. The linear dimensions of the nucleotides $R = 10^{-7}$ centimeters. The diameter of the nucleus is five microns. The number of particles in the chain is 10^5 ; the number of chains is 10. The binding energy is of the order of 1 ev; in order to satisfy the inequality (5), let us use a value of $U_1^{(1)} = 1.2$ ev. Then, we obtain for the reduplication time

$$t^* = 107 \text{ seconds} \quad (6)$$

In order to find the first approximation it is necessary to know the depth of the other "foreign", that is in contrast to "their own" pockets.

Evidently, $|U_1^{(2)}| < |U_1^{(1)}|$. It turns out that if we assume that $U_1^{(2)} = 0.54$ ev, the time t^* that is obtained is the same as for (6).

7. Further investigation of the relationship t^* to the parameters $U_1^{(1)}$, $U_1^{(2)}$ and $C-N$ has led to the following results (see Fig. 3 and Table).

In addition to the critical value for the depth of their own pocket, determinable by (5), a critical value exists for $U_1^{(2)} = U^{(2)}$, such that with all values of $|U_1^{(2)}| > |U^{(2)}|$ the time needed for building the chain becomes infinite. This is associated with the fact that the particles are held too firmly in the "foreign" pockets. Therefore, their own pockets should not only be sufficiently deep but the "foreign" pockets should be sufficiently shallow. If the depth of the foreign pocket is less than the critical one, the time for building the chain is finite and, as it appears, ^{practically} does not depend on the depth of the pocket. As is seen from the Table and Fig. 3, in case $C-N = 0$ the critical value for the foreign pocket, equal to 0.54 ev, where t^* equals 107 seconds does not correspond to the critical value of the depth of their own pocket, 1.15 ev. In case $C-N = 9$ their own pocket should be deeper than 1.09 ev, and the foreign pockets should be no deeper than 0.54 ev, where $t^* = 16$ seconds. Increase in the excess of particles reduces the reduplication time and changes the critical energy values.

8. Therefore, it has been shown that within the framework ^{of} a simple physical model of the second stage of reduplication of chain molecules it is

possible to investigate the kinetics of this process mathematically. In order to understand the theoretical course of the process no assumptions are required as to the nature of any of the specific attraction forces between the nucleotides and the DNA molecule. We assume only that the energy of these attractions, of the order of 1 ev, varies by approximately two times for their own and for the foreign nucleotides. The model proposed makes it possible to estimate the time during which the second stage of reduplication occurs. This time is of the order of minutes, which is in agreement ^{with} (and in any case no more than) certain experimental data concerning the interphase time. Consideration of the fact that four rather than two kinds of particles participate in the construction of DNA actually leads to an increase in t^* and to more fixed limits for the binding energies in the foreign and in their own pockets. This ^{circumstance} explains, possibly, the fact that only four kinds of nitrogen bases having suitable binding energy values participate in the construction of DNA. We should like to note that the model provides a perfectly identical reproduction of the order of arrangement of the nucleotides in the new and in the old chains but in principle permits a disruption of this order ("spontaneous mutations") with a probability proportional to $\exp -U^{(1)} \frac{1}{kT}$.

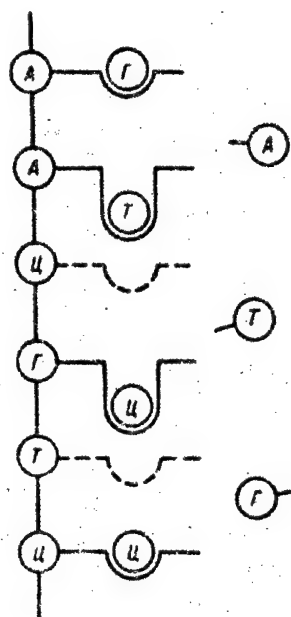


Fig. 1. Diagram of section of DNA chain in the course of sorption of free nucleotides during reduplication.

The deep pockets correspond to "their own" particle (thymine (T) near adenine (A) and cytosine (C) near guanine (G); the small pockets, to the "foreign" particle (guanine near adenine and cytosine near cytosine).

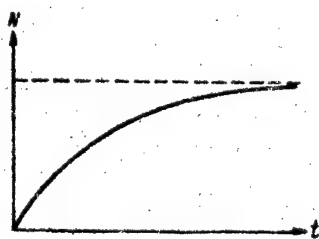


Fig. 2. Schematic representation of the relationship of the number of pockets occupied by "their own" particles to the time.

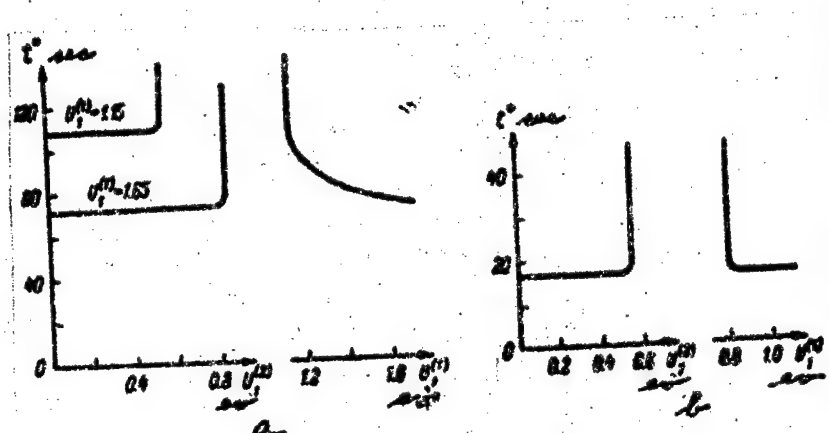


Fig. 3. Relationship of the reduplication time t^* to the depth of the "foreign" potential pocket (left-hand portions of the graphs) with fixed values for the depth of "their own" pockets $U(1)$ and the relationship t^* to the depth of "their own" $U(2)$ pockets (right-hand portion of the graphs) with a depth of the "foreign" pocket of $U(1) < U(2)$

a) excess of nucleotides C - N = 0; b) excess of nucleotides C - N = 9.

Table

Relationship of the Time of Reduplication t^* and the Maximum Depth of the "Foreign" Potential Pocket $U(2)$ to the Excess of Nucleotides C - N and to the Depth of "Their Own" Potential Pocket $U(1)$

	C-N				
	0	9	99	999	
	$U(1)$ (in μm)				
	1.15	1.65	1.09	1.83	0.878
t^* (in sec.) . .	1.07	71	16	3	0.5
$U(2)$ (in μm) . .	0.54	0.84	0.54	0.5	—

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Cytological Study of the Various Stages of the Life Cycle of Gregarines from

Dragon-Fly Larvae

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In a number of works of various authors the dynamics of nucleic acids, polysaccharides and other cytoplasmic inclusions of ~~protosans~~ at various stages of the life cycle have been shown. Among these works mention should be made of the investigations of Ye.A. Shubnikova (1947) and Ye.N. Gromova (1948b), who showed definite dynamics of nucleic acids in Paramecium caudatum; Gromova (1948a), who observed age changes in the content and distribution of nucleic acids in Bursaria truncatella; as well as those of Schwartz (1956), who studied ribonucleic acid in P. bursaria. Patillo and Becker (1955), Ye.M. Kheysin (1958) and others investigated the cytochemical changes in the life cycle of coccidia.

The changes in different substances in the life cycle of gregarines have been studied little to date. Giovannola (1934) determined the presence of glycogen in the ~~vegetative~~ forms of certain monocystic and polycystic gregarines. Ganapati and Narasimha-Murti (1955) described the dynamics of the polysaccharides in the life cycles of gregarines from the group of ~~myriapods~~ Loubatières (1955), in an extensive work devoted to classification and morphology of monocystic gregarines of the group of oligochete worms, used certain cytochemical methods for the study of the nuclear structure.

In the present work we have set before ourselves the problem of studying

the dynamics of the content of nucleic acids, polysaccharides, protein and fat inclusions, "volutin" and mitochondria at various stages of the life cycle of polycystic gregarines by means of cytochemical methods.

Material and Methods

For the investigation two species of gregarines from the family Actinocephalidae were used -- Geneiorhynchus aeschnae Crawley and Hoplorhynchus ligocanthus (variety Siebold) which parasitize dragon-fly larvae of various suborders (Aeschna sp. from Anisoptera and Coenagrion sp. from the suborder Zygoptera). Both species proved to be very similar in the cytochemical respect. Therefore the material is being presented chiefly for Geneiorhynchus aeschnae, and the data for Hoplorhynchus are brought in for comparison. The entire material was collected at the Peterhof Biological Institute of Leningrad University.

The vegetative forms of gregarines were fixed in smears or along with sections of the host intestine. For the purpose of obtaining the stages of development and the mature oocysts the gamontocysts which were excreted into the water along with the larval excrement were put into salt cellars and microaquaria, and they were fixed during the course of development at various time intervals. (The names oocyst and gamontocyst correspond, respectively, to "spore" and "cyst" in the old terminology). Depending on the temperature the mature oocysts are formed three to seven days after the experiment is begun.

For the purpose of fixing the smears Schaudinn's and Nissenbaum's fluids were used (saturated solution of mercuric chloride, glacial acetic acid, 40 percent formalin, butyl alcohol in a proportion of 10:2:2:5). The latter fixative

possesses a certain advantage over the Schaudinn's fluid, namely, it causes the oocysts to stick better to the glass slide. For the purpose of fixation of the individual gregarines, gamontocyst and sections of intestine of the infested larvae use was made of the following fixatives: Helly's mixture (Zenker-formol), Carnoy's fluid, Serr's fluid (absolute alcohol, formalin, glacial acetic acid in a proportion of 6:3:1), and Champi's fluid. Ten-percent neutral formalin and ~~calcium-cobalt-formalin~~ fixative made according to the McManus method with subsequent potassium bichromate treatment were used for staining the lipids with Sudan black B (Pierce, 1956). The intestinal sections were embedded in paraffin using xylol or benzol, and various gregarines and gamontocysts were embedded in celloidin plates according to the method of Peterfi.

We determined the desoxyribonucleic acid (DNA) by means of the Feulgen reaction, utilizing preparations treated with the enzyme desoxyribonuclease for the purpose of control. For the purpose of determining the ribonucleic acid (RNA) staining with methylgreen-pyronine was used at a pH of 4.7, and toluidine blue was used according to the Brachet method (1953). Control sections were treated with a solution of crystalline ribonuclease or were hydrolyzed 10 minutes at 60° in one N HCl (Vendrelly, 1949). (The enzymes desoxyribonuclease and ribonuclease were obtained from the laboratory of cell biochemistry of the Institute of Cytology of the Academy of Sciences USSR). The material which stopped being stained by pyronine and toluidin blue after the effect of ribonuclease and HCl was taken for RNA. The same results were obtained after treatment with ribonuclease and HCl. The "volutin" was stained with methylene blue according to the method of Mayer

(Roskin and Levinson, 1957) or with the Schiff reagent without preliminary hydrolysis (Reichenow, 1928).

The polysaccharides, mucopolysaccharides and the mucoproteins were demonstrated by means of the PAS method after McManus and Hotchkiss in the Lilly modification (Glick, 1950); in addition, for the purpose of detecting the glycogen use was made of Lugol's staining fluid and the method of Bauer-Schiff. The control sections were treated with saliva (one hour at 37°), with cold and with hot water. For the purpose of determining the acid mucopolysaccharides a staining with 0.5 percent aqueous solution of toluidine blue (Pierce, 1956) and the Hale method with dialyzed iron were used (Hale, 1946). However, as has been shown (Pierce, 1956; Kheysin, 1958), the latter method is not specific and by means of it both acid and neutral mucopolysaccharides can be shown as well as various proteins. The PAS method in combination with preliminary processing of the sections with hyaluronidase was used for determining the hyaluronic acid. We demonstrated proteins with a mercuric chloride solution of bromphenol blue (Maxia, Brewer and Alfert, 1953) and by means of the Schiff ninhydrin method (Yasuma and Ichikawa, 1953); at a pH of 8.05 we demonstrated the basic proteins of the histone type with fast green (Alfert and Geschwind, 1953). In the determination of the proteins sections treated with pepsin (two hours at 37°) served as control. The bromphenol blue and the Schiff ninhydrin reagent generally gives the same results, but in the latter case a considerably weaker staining is obtained. The lipids were stained with Sudan III, and the phospholipids, glycolipids and lipoproteins with Sudan black B according to the McManus method (Pierce, 1956). Sections which were

First treated with chloroform (two to 2.5 hours at 37°) served as a control. For the purpose of demonstrating the mitochondria staining with acid fuchsin was used according to Kuhl and Altmann method (Romeys, 1953).

Life Cycle of Gregarines

The life cycles of Genetorhynchus aeschnae and Hoplorhynchus oligocanthus are very similar to the life cycles of species such as G. monnieri (Gal'tsov, 1911) and Actinocephalus parvus (Weschenfelder, 1938). The vegetative forms of the gregarines investigated (Fig.1) live in the center portion of the intestine of dragon-fly larvae. Solitary gregarines were encountered in the intestine of adult dragon flies of Aeschna sp. and Libellula sp.

The life cycle of G. aeschnae is represented schematically in Fig.2. Sporozoites hatch out of the oocysts swallowed by the dragon-fly larva. The sporozoite embeds itself with its anterior end into the epithelial intestinal cell and is converted into a trophozoite. At first, the young trophozoite is still without any epi- and protomerite. Its cytoplasm is markedly vacuolated, while the nucleus, which is large by comparison with the size of the entire trophozoite, contains several nucleoli. With its growth first an epi- and then a protomerite are successively differentiated in the trophozoite. The gregarines remain attached to the epithelium until the end of their growth period. After this, they break off, and are found in the intestinal lumen in the free state before the beginning of encystment. Such gregarines devoid of epimerites have the name of "gamonts". Before the beginning of cyst formation two gamonts adhere to each other closely and form a syzygy.

The stage of syzygy is very brief and probably for this reason P. Gal'tsov (1911) was unable to find it in the development of G. monnieri. The partners elaborate a common capsule, as a result of which a gamontocyst occurs. The asexual portion of the gregarine life cycle is completed at this stage, and the gamontocysts enter the water along with the larval excrement where sexual multiplication is accomplished. The formed gamontocysts ^{are} surrounded by a double membrane, and nuclear division begins in it which precedes the formation of gametes. During division one ^{always} gamont/outstrips the other somewhat. The nuclei of the future gametes contain four chromosomes and are haploid. The formation of gametes is accompanied by the disappearance of a partition between the gamonts in the cyst. Apparently, copulation of the gametes occurs at different times, because the oocysts in the gamontocysts are at different stages of development. As a result of gamete copulation a zygote is created.

The zygote, which is at first uniformly ovate, gradually assumes its final oocyst shape. In G. aeschnae the oocytes are spindle-shaped, while in H. oligocanthus they have a half-moon shape. The ^{residual} body is evidently absorbed with the development of the oocysts. In the gregarines investigated only the zygote is diploid (eight chromosomes). At the first division of the synkaryon a chromosomal reduction occurs, and the nuclei of the sporozoites which arise as a result of three metagametic divisions are again haploid (four chromosomes). At the time of maturation the membrane of the gamontocysts breaks, and the oocysts pour out into the water, where they are swallowed by the dragon-fly larvae. The life cycle of the gregarines then begins again.

Under field conditions it was possible to establish the existence of relationship of the life cycle of the parasites to the life cycle of their hosts. Usually, the small and moderate-sized dragon-fly larvae are maximally infested, whereas the large larvae which are ready for metamorphosis are, as a rule, without any gregarines. In very rare cases, solitary gregarines are encountered in adult dragon flies. In June-July under conditions of Leningradskaya Oblast' a mass flight of dragon flies occurs, and precisely at this time it is possible to find the largest number of gamontocysts in the larval excrement.

The preparation for metamorphosis is associated with physiological changes in the bodies of the larvae: the musculature of their bodies and intestinal walls become sluggish; there is no food in the intestine. The changes in the host organism, in their turn, influence the gregarines, stimulating their encystment and their coming out into the water. The gregarines which enter the adult dragon flies probably are doomed to destruction, because even in the event of encystment the oocytes cannot continue their development outside of a water body. Cases of direct entrance of gregarines from the larvae into the adult Agrionidae dragon flies during metamorphosis are known in the case of Actinocephalus sieboldii (Foerster, 1938).

Cytochemical Investigations

Nucleic acids. In the gregarines investigated desoxyribonucleic acid is present at all stages of the life cycle (Fig.3). At the periphery of the nucleus of a mature sporozoite a ring is stained with methygreen, which is slightly thickened on one side. The results of staining permit us to speak of the presence

of DNA in the sporozoite nucleus. From the time of formation and growth of the trophozoites up to the beginning of nuclear division DNA is detected with very great difficulty in the gamontocysts: at the periphery^{of} the nucleoli comparatively small and few Feulgen-positive granules may be discerned. Staining of the granules is exceptionally weak. In the gamontocysts Feulgen-positive granules become progressively more distinct (six to eight granules in the resting nuclei), and during division the entire DNA is concentrated in the chromosomes. A particularly bright coloration is observed at the beginning of the formation of gamete nuclei and during their confluence into a synkaryon. Afterwards, the DNA is continuously present in the oocyst nuclei and in the chromosomes during division of these nuclei, and then in the sporozoite nuclei which are first compact and are Feulgen-positive as a whole. In the mature sporozoites a redistribution of the DNA occurs in the nucleus, and the latter is located at the periphery of the nucleus.

Ribonucleic acid is also present in all stages of the life cycle, but its dynamics are different from the DNA dynamics (Fig.4).

While the RNA was found in the sporozoite cytoplasm in very small quantities with the growth of the trophozoite its quantity increases progressively, and at various stages the RNA is concentrated in different parts of the gregarine. In the young trophozoite the RNA predominates in the epimerite and in the nucleoli (they are most intensely stained with pyronine and toluidine blue). In the formed trophozoites^{the RNA is predominant}-- in the deutomerite and nucleoli. After processing with ribonuclease or HCl the staining completely disappears from the young trophozoites, and in the

mature trophozoites a certain quantity of pyroninophilic granules, apparently unassociated with RNA, ^{is} preserved in the cytoplasm, particularly in the ectoplasm. With the conversion of trophozoites into gamonts the quantity of RNA in the cytoplasm decreases notably, and the pyroninophilia becomes very weak. In the nucleoli the RNA remains unchanged. The same picture is observed also during the union of gamonts in syzygy. During the period of sexual multiplication RNA is present in the cytoplasm in an insignificant quantity. The division of nuclei in the cyst is accompanied by the disappearance of nucleoli and the further reduction in the quantity of RNA in the cytoplasm.

Protein inclusions. During the course of the entire life cycle of gregarines the proteins remain essentially unchanged. In very young trophozoites they are diffusely distributed in the cytoplasm of the epi- and deutomerite, whereby in the latter individual granules also occur. In the nucleus they are present in the karyoplasm and in the nucleoli. In the well-formed trophozoites the quantity of protein granules increases in the cytoplasm, and the nucleoli stain particularly intensely along the periphery. In the mature gamonts the intensity of staining is reduced slightly by comparison with the trophozoites because of a certain reduction in the quantity and size of the cytoplasmic granules. In the gamontocysts the proteins are included in the nuclei, forming nucleic acid-protein complexes, as a result of which the Feulgen-positive granules and frequently the nucleoli stain a bluish-violet color with methylgreen-pyronine. During nuclear division the proteins are included in the chromosomes and are

present in an insignificant quantity in the cytoplasm of the gamonts, in the residual body, in the well formed oocysts, in the cytoplasm and nuclei of the sporozoites.

The histones are distributed in accordance with the localization of nucleoproteins in the nucleus of the periphery of the nucleoli and to a lesser degree in the central portion of the nucleoli and ⁱⁿ the karyoplasm; in the cytoplasm, they are present in the granules filling the proto- and deutomerite.

Polysaccharides (Fig.5). The accumulation of polysaccharides in the gregarines investigated begins very early, when the young trophozoite is ^{not as yet} completely differentiated. In such gregarines granules appear in the epimerite and in the perinuclear zone in the deutomerite which stain brightly with the PAS method but weakly with the Bauer-Schiff method. The intensity of staining is different: along with the bright granules there are also weakly stained granules. Preliminary treatment with saliva, cold and hot water does not remove the stain.

According to the data of Ganapati and Narasimha-Murti (1955) and other authors, one of the varieties of glycogen-paraglycogen may be deposited in the gregarines as reserve substance.

The paraglycogen is not digested by saliva, is poorly soluble in water, and is stained a brown color with iodine. With growth the quantity of paraglycogen in the cytoplasm increases progressively, so that the entire cytoplasm is filled with large granules in the gamonts. With staining the granule sizes of the gamont can be divided into two groups. The first group consists of gamonts with large (1.5-3 microns) granules which fill the entire ^{endoplasm} and not uncommonly are confluent.

The granules are spherical with a very bright center and a clearer peripheral zone. In the gamonts of the second group the granules, although they have the same structure, possess smaller dimensions (1-1.2 microns) and are stained considerably less intensely, as a result of which the general staining produces the impression of being less. We are inclined to regard the differences found in the size, staining and apparently in the quantity of granules in G. aeschnae as a manifestation of sexual dimorphism. In H. oligocanthus we did not find any such differences, although Muhl (1921) indicates the presence of sexual dimorphism in this species. Differences in the content of granules of paraglycogen in the male and female gamonts of Grebneckiella pixallae from Scolopendra morsitans have been noted by Ganapati and Narasimha-Murti.

The gamonts which have combined into a cyst are very rich in paraglycogen, which is used up during the course of development. In connection with this, the structure of the granules is changed in the developing gamontocysts: they lose their clarity of outline and are surrounded by diffusely stained area. The impression is created that the granules dissolve. Along with a reduction in the size a certain reduction in the number of granules occurs in the cyst beginning with the center and going toward the periphery. In the developing oocysts of H. oligocanthus from six to 10-12 paraglycogen granules are found, and quite a lot of paraglycogen is preserved also in the residual body. In the formed oocysts of G. aeschnae there is very little polysaccharide: the cytoplasm of the sporozoites and small areas at the poles of the oocytes and in the area of the sporozoite nucleus give a weak PAS-positive reaction. In the latter case, the staining is probably not

associated with polysaccharides but rather with the presence of compounds of a phospholipid type in the nucleus. This supposition is probable, because we have found similar compounds in the gamont nuclei.

In staining gregarines by the toluidine blue method according to Brachet we directed attention to the fact that with the growth of trophozoites the metachromasia increases in their cytoplasm; this is not removed by ribonuclease treatment. Study of this phenomenon has shown that even in the trophozoites consisting only of epi- and deutomerite red and purple granules (γ - and β -metachromasia) begin to appear in the cytoplasm of the latter. The quantity of γ -metachromatic granules increases in parallel with the growth of trophozoites and becomes maximal in the gamonts. These granules are scattered throughout the entire cytoplasm, but the principal accumulations are observed in the ectoplasm of the proto- and deutomerite, in the neck of the epimerite and in the epimerite itself, where accumulations form transversely arranged sections. Pierce believes that γ -metachromasia can be produced by the presence of sulphuric acid esters (acid mucopolysaccharides). The results of staining for mucopolysaccharides according to the Hale method and for metachromasia proved to be the same. It was impossible to determine specifically how the mucopolysaccharides are localized in the gregarine ectoplasm. In any case, hyaluronic acid is not included among them, because treatment with the enzyme hyaluronidase does not reduce the intensity of staining. With the formation of the gamontocysts acid mucopolysaccharides participate in the elaboration of its membrane.

β -metachromatic granules are particularly numerous in the endoplasm of large trophozoites. In the gamonts the quantity of these granules is reduced, and the endoplasm stains diffusely. Apparently β -metachromasia can be associated with the presence of mucoproteins which do not dissolve in saliva and which give a PAS-positive reaction.

Lipids. In connection with lipids stainable with Sudan III, the trophozoites show a marked individual variability (from an almost complete absence to an accumulation of fat droplets in the endoplasm of the proto- and deutomerites). This variability is probably depends on the physiological state of the host and on the degree of its nutrition. However, in general with growth an accumulation of fat occurs. The comparatively large fat droplets are stained first in the cytoplasm of the gregarines with Sudan black; secondly, the ring-like structures which constitute the mitochondrial membranes. In the nucleus the nucleoli are diffusely stained (the nucleoli are diffusely stained also by the PAS-method). According to Pierce, staining with Sudan black in combination with positive PAS-reaction in the absence of hydrolysis in saliva and in hyaluronidase is evidence of the presence of compounds of ^{the} phospholipid, glycolipid or lipoprotein type. After treatment with chloroform the staining is completely removed from the cytoplasm and individual poorly stained granules are preserved in the nucleoli. In the mature gamonts the distribution of lipids is not changed by comparison with the trophozoites.

The mitochondria form small accumulations of rounded or ring-like

bodies in the endoplasm of the proto- and deutomerite of the trophozoites and gamonts.

"Volutin". After staining with Schiff reagent without hydrolysis Reichenow (1928) observed bright granules in the cytoplasm of gregarines which he designated volutin granules. Using Reichenow's method and the method of Mayer we obtained the following results. After staining according to the Reichenow method young trophozoites remained colorless, while a very weak diffuse staining appeared in the large trophozoites. By the Mayer method small granules are stained in the cytoplasm of the epi- and deutomerite of the young trophozoite. The volutin granules, similar to what was described by Reichenow, could not be found.

The results of cytochemical investigation of a life cycle of gregarines are presented in Table 1.

Discussion

On the basis of the data obtained it seems possible to us to characterize the physiological significance of the individual stages in the gregarine life cycle in the following way.

1. Sporozoite -- a very brief stage, accomplishes the colonization and attachment of gregarines in the new host. Polysaccharides (paraglycogen) are the energy material which provides for the movement of the sporozoites.

2. Trophozoite -- ^{growth} stage and stage of accumulation of substance by feeding. This stage assures the subsequent process of sexual multiplication. Therefore, an accumulation of RNA, proteins and polysaccharides

(possibly also fats) occurs in the trophozoite. During the process of growth of the trophozoite the functional significance of its various parts changes. Thus, in the young trophozoites the epimerite functions most actively, and through it the nutrition of the gregarine is accomplished from the epithelial cells of the host. Therefore, RNA and polysaccharides are primarily present in the epimerite. With the growth of the trophozoite the epimerite is converted progressively into an anchor, which holds the gregarine in the attached state, while the function of nutrition probably begins to be accomplished by the deutomerite which is in contact with the intestinal contents. In connection with this, the changes in the distribution of RNA and in the polysaccharides which begin to predominate in the deutomerite become understandable. It may be supposed that the completion of synthesis of substances necessary for the development also leads to the separation of the trophozoite from the intestinal wall, as a result of which it becomes a gamont. In the gamont there are no active synthetic processes, and probably for this reason the quantity of RNA is reduced in its cytoplasm.

3. Gamontocysts -- the stage which carries out two functions: a) providing for the development of gametes and the formation of the colonization stage -- oocysts with sporozoites through substances previously accumulated; and b) protection of the developing elements against harmful influences of the environment with the aid of a membrane which includes acid mucopolysaccharides.

4. Oocysts -- the stage which provides for the colonization of the species and which protects the sporozoites from death from the influence from environmental factors.

The protection of DNA at the periphery of the nucleoli is of great interest. Judging by the existing data the absence or presence of DNA in the nucleoli depends on the biological object. Thus, Loubatières (1955) showed that in the nucleus of the monocystic gregarine Zygocystis grassei the nucleolus is stained intensely according to the Feulgen method, and only individual granules in it are stained with Light Green, while in Gregarina polymorpha from the meal-worm the entire nucleus, with the exception of the nucleolus stains by the Feulgen method (Jirovec, 1927). In the nucleoli of the macronucleus of Paramecium bursaria Feulgen-positive chains of granules have been described (Schwartz, 1958).

With respect to the content of DNA in the nucleoli of multicellular animals there is no agreement. DNA has been found in isolated liver nucleoli of the rat (Downs, 1957), in the nucleoli of mollusc oocytes of *Mytilus* and *Aplysia* and in the vacuols of ^{the} nucleolus in *Haliotis* (Bolognari, 1957), in cultures of chick fibroblasts (Lettré, 1955). At the same time Swift (1953), contradicting Downs, believes that the nucleoli of the rat liver nuclei are Feulgen-negative and do not contain DNA. B.V. Kedrovskiy (1951) believes that DNA attaches to the nucleolus from the periphery in the form of a solid layer of different degrees of thickness or in the form of individual granules and only rarely is included in the nucleolus itself.

The DNA granules at the periphery of the nuclei of the gregarines which we investigated give a weak Feulgen-positive reaction and do not stain with methylgreen. We believe that in the vegetative stages of the gregarines the DNA is found in the depolymerized state. Downs, referring to a number of works, pointed

out that depolymerized DNA is stained pink with pyronine, whereas highly polymerized RNA is stained blue or purple (intranucleolar granules of trophozoites and gamonts). Regular changes in the quantity of DNA during the ontogeny of Bursaria truncatella have been described by Ye.N. Gromova (1948a). During the first few hours after division of infusoria she observed the minimum quantity of DNA in the macronucleus. Then the quantity of DNA gradually increases and becomes maximal before a new division.

Definite dynamics in the cytoplasmic RNA associated with age changes have been established for G. aeschnae and H. oligocanthus. As has been mentioned, there is most RNA in the cytoplasm of young differentiating trophozoites. After the completion of differentiation and with growth the pyroninophilia gradually decreases, and before cyst formation becomes minimal in the mature gamonts. A slight degree of pyroninophilia is preserved throughout the sexual process, and then in the oocysts and sporozoites. In B. truncatella Ye.N. Gromova also observed age changes in the concentration of RNA. Probably, Gromova's work was unknown to B.V. Kedrovskiy, who in 1951 described the absence of data concerning age changes in RNA in protozoans. A.A. Savinovskaya (1952) expressed the idea that a direct relationship exists between the size of the nucleoli and the quantity of RNA in the cytoplasm. The existence of such a relationship might serve as an indirect proof of the fact that the nucleolus participates in the synthesis of RNA. In contrast to cells of multicellular animals the growth of G. aeschnae is accompanied by an increase in the average size of the nucleoli and a reduction in the quantity of RNA in the cytoplasm. Probably, in this case the role of the

nucleoli in the synthesis of RNA is insignificant. In Table 2 an increase is shown in the diameter of the nucleoli with the growth of the gregarines.

In the growing macrogametes of the coccidian Eimeria intestinalis an increase occurs in the size of the nucleoli, and in the mature gametes a reduction in their size is observed, which is explained by reduction of the synthetic activity of this stage of the cycle (Kheysin, 1958). In the trophozoites and gamonts of G. aeschnae the number of nucleoli does not increase, but the increase in the size of the nucleoli is apparently associated with synthetic processes occurring in the cytoplasm (synthesis of polysaccharides, mucoproteins and, perhaps, fats).

Caspersson (1950) and Brachet (1955) believe that those cells are particularly rich in RNA which actively synthesize protein. As far as can be judged by the results of staining the trophozoites containing considerable RNA are also rich in proteins. At the same time, the reduction in the RNA content in the cytoplasm of gamonts is not associated with a reduction in the quantity of protein granules. On the other hand, we believe that with the growth of trophozoites an increase occurs in the synthesis of such proteins as mucoproteins. This may be judged by the intensification of the PAS-reaction and in the β -metachromasia with toluidine-blue staining. True, β -metachromasia can also^{be} produced by highly polymerized carbohydrates or compounds containing phosphoric acid aside from the mucoproteins. The PAS-reaction is not eliminated by preliminary treatment with saliva, hyaluronidase and ribonuclease. An accumulation of

mucoproteins in the cytoplasm of macrogametes of coccidians in parallel with the reduction of RNA in it was observed by Ye.M. Kheysin (1958).

The accumulation of paraglycogen with the growth of the gregarines ^{has been} described more than once (Giovannola, 1934; Ganapati and Narasimha-Murti, 1955; Leubatières, 1955 and others). The majority of authors notes that most often the glycogen is deposited in the deutomerite and is absent from the protomerite. This is well known for G. cuneata and G. polymorpha from meal-worms (Muhl, 1921;

Giovannola, 1934), for Monocystis sp. from the earthworm (Giovannola, 1934), for Grebneckiella pixellae from Myriapoda (Ganapati and Narasimha-Murti, 1955).

In G. aescnae and H. oligocanthus the granules of paraglycogen are deposited both in the deutomerite and in the protomerite, whereby in certain cases the staining is brighter in the protomerite than in the deutomerite. The presence of glycogen in the sporozoites is known for monocystis sp. (Giovannola, 1934) and the coccidians Eimeria intestinalis (Kheysin, 1958).

The question of the nature of "volutin" remains unclear to date. Muhl (1921) and Reichenow (1928) demonstrated volutin in the endoplasm of Gregarina polymorpha in the form of large granules. Through the example of the flagellate Haematococcus it was shown that phosphorus is included in volutin. When there is an abundance of phosphorus in the nutrient solution an accumulation of volutin occurs in the flagellates, whereas in a medium free of phosphorus the volutin is used up rapidly and ^{is} not replaced (Reichenow, 1928). Van den Berg (1946) and Belozerskiy, according to Roskin and Levinson (1957) believe that volutin basically consists of RNA, which is distinguished from the ordinary RNA by its

more complex composition. According to Pierce, the capacity of being stained with the Schiff reagent without preliminary acid hydrolysis is characteristic of the cytoplasmic reaction associated with the presence of acetalphosphatides, which is usually in a combination with phosphatides. Therefore, the presence of a cytoplasmic reaction as well as the capacity of being stained with Sudan black and by the PAS method characteristic of phosphatides permit us to speak of the fact that inclusions containing phosphorus are present in the cytoplasm of the gregarines being investigated. Apparently, they represent/so-called "volutin".

As has already been mentioned, in the trophozoites and gamonts of the gregarines investigated lipids were found in the nucleoli which were extractable with chloroform. Callen and Tomlin (1950) believe that sometimes the capacity of the peripheral layer of the nucleoli of amphibian oocytes for being stained with Sudan black is associated with the presence of lipoproteins. The lipids and lipoproteins in isolated nuclei of liver cells of the rat were described by Downs (1957). He pointed out that about three to 10 percent was made up of phospholipids contained chiefly in the nucleoli.

Resumé

At all stages of the life cycle of the polycystic gregarines Geneiorhynchus aeschneae and Hoplorhynchus oligocanthus both DNA and RNA were found. Dynamics associated with age changes are characteristic of the RNA: most of the RNA is in the cytoplasm of the young trophozoites; with the growth of the gregarine the quantity of the RNA is decreased, and in the mature gamonts before cyst

formation it becomes minimal. Proteins are present at all stages of the life cycle without any essential changes. The polysaccharides appear in the cytoplasm of very young trophozoites. With the growth of the latter the quantity of polysaccharides increases progressively and becomes maximal in the gamonts. During the course of sexual multiplication the polysaccharides are used up, so that in the sporozoites they are preserved in an insignificant quantity necessary for accomplishing the basic function of the sporozoites -- colonization and attachment in the intestine of the new host. Differences in the quantity and size of granules of polysaccharides in the cytoplasm of G. aeschnae gamonts permit us to speak of the existence of sexual dimorphism in this species. Along with polysaccharides of the glycogen type (or paraglycogen) the presence of acid mucopolysaccharides as well as mucoproteins, lipids and compounds containing phosphorus (the so-called "volutin") has been noted in the trophozoites and gamonts.

As a result of the cytochemical study the physiological differences were shown in various stages of the life cycle of gregarines. It was noted that representatives of various genera -- G. aeschnae and H. oligocanthus, are very similar in a cytochemical respect despite the fact that they ^aparasitize representatives of different suborders. Apparently, this similarity of gregarines in this case is a reflection of a similarity in the biology and ecology of the hosts.

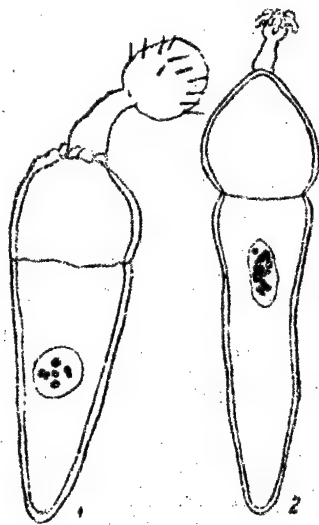


Fig. 1. Gregarine trophozoites *Geneiorhynchus aeschnae* (1) and *Hoplorhynchus*.

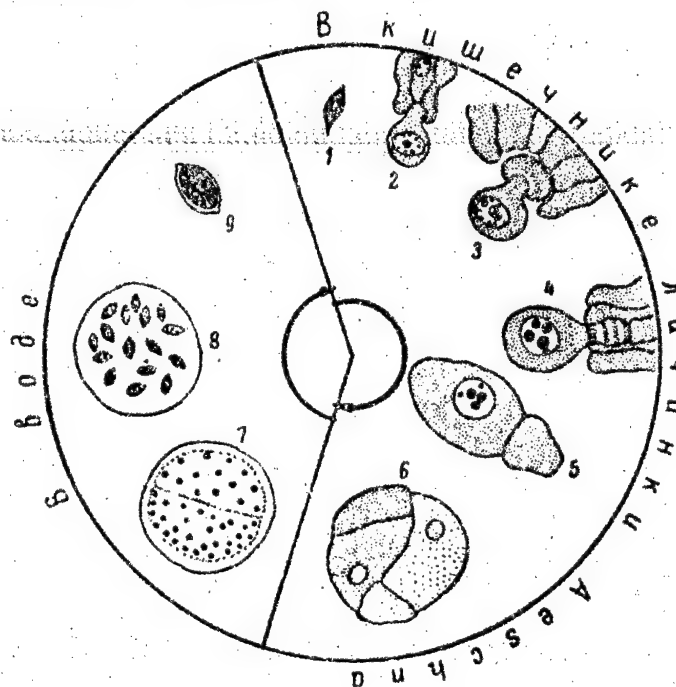


Fig. 2. Life cycle of *geneiorhynchus aeschnae*. 1) sporozoite; 2- 4) formation of trophozoite; 5) gamont, c) zygote; 7) gamontocyst with large number of nuclei; 8) gamontocyst with oocysts; 9) oocysts with sporozoites. 1-6) in the intestine of the dragon-fly larva; 7-9) in the water.

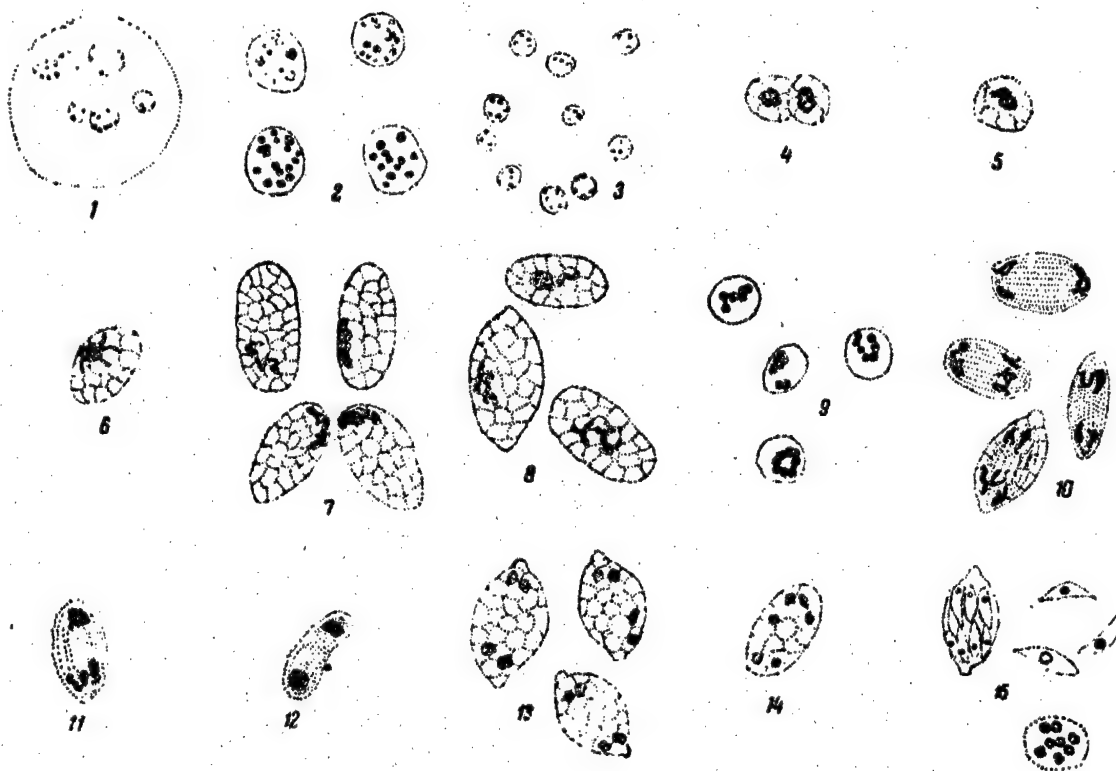


Fig. 3. DNA at various stages of the life cycle of *geniorhynchus aeschnae*. 1

- 1) nucleus of trophozoite or gamont (objective 40x; aperture, 0.65; ocular, 15x);
- 2) nuclei of gamontocysts at the beginning of progamous division;
- 3) nuclei of gamontocysts at the end of progamous division;
- 4) copulating gametas;
- 5) zygote; 6) prophase; 7-9) metaphase; 10-11) anaphase;
- 12) telophase of the first zygote division; 13) oocysts after second metagamous division; 14) oocysts after third division of part of nuclei; 15) mature oocysts and sporozoites (objective, 90x; aperture, 1.25; ocular, 15x).

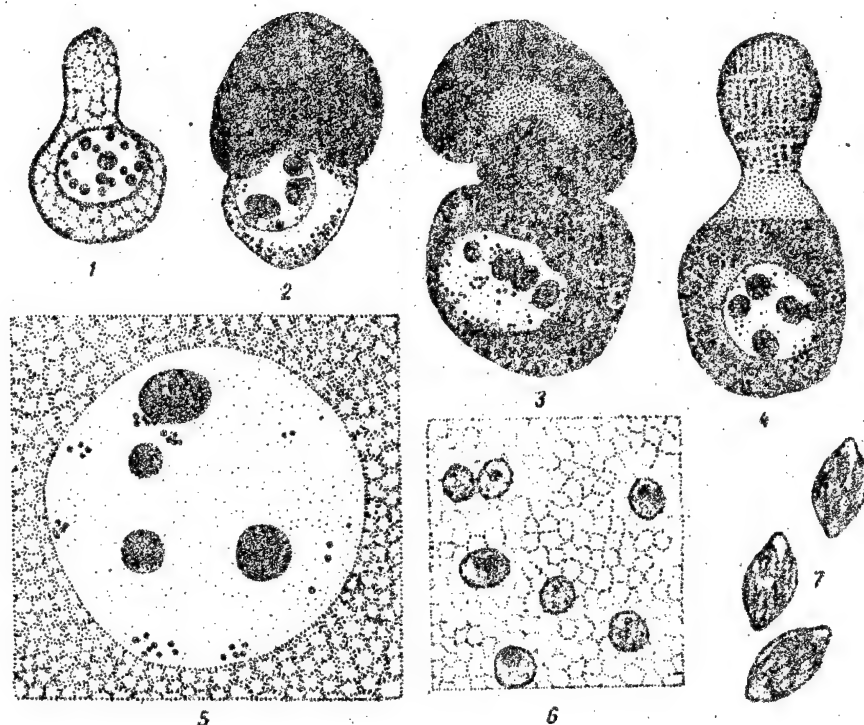


Fig. 4. RNA at the various stages of the life cycle of *geniorhynchus aeschnae*.

- 1-4) in the nucleus and cytoplasm of trophozoites;
- 5) in the nucleus and part of the cytoplasm of the gamont;
- 6) in the nuclei and cytoplasm of the gamontocysts;
- 7) in the oocysts with sporozoites (objective, 90x; aperture, 1.25; ocular, 15x).

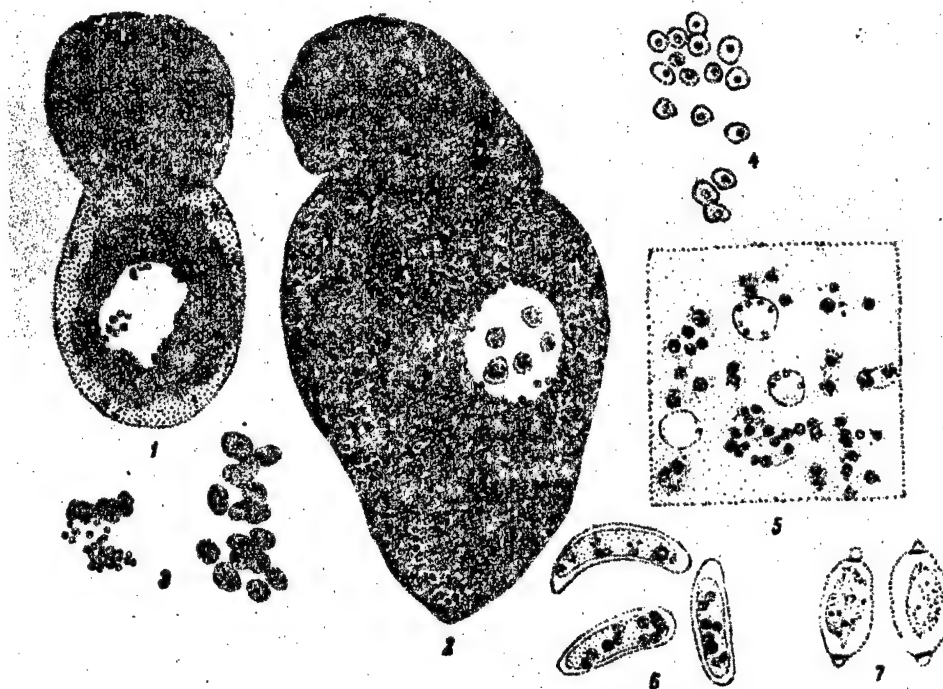


Fig. 5. Paraglycogen at various stages of the life cycle of *geneiorhynchus aeschnae* and *hoplorhynchus oligocanthus*.

- 1) in a young *G. aeschnae* trophozoite;
- 2) in a *G. aeschnae* gamont;
- 3) granules of paraglycogen from heterosexual gamonts of *G. aeschnae*;
- 4) granules of paraglycogen from the *G. aeschnae* gamontocysts;
- 5) paraglycogen in the *H. oligocanthus* gamontocysts;
- 6) granules of paraglycogen in the *H. oligocanthus* oocysts;
- 7) paraglycogen in the oocytes of *G. aeschnae* (objective, 90x; aperture, 1.25; ocular, 15x).

Table 1

Nucleic Acids, Proteins, Polysaccharides and Lipids
at Various Stages of the Life Cycle of the Gre-
garine *Genetiorhynchus Aeschnae*

1 Стадии и структуры	2 Вещества	3 Липиды									
		3 ДНК	4 РНК	5 Белки	6 Гистоны	7 Парагликоген	8 Кислые мукополисахариды	9 Нейтральные жиры	10 Липиды	11 Соединения типа фосфо- липидов	12 Липиды
13 Споро- зоит	Цитоплазма	+	+	±		±					
	Ядро	+	+	+		+					+
14 Молодой трофо- зоит	Цитоплазма эн- мерита	-	+	+		+	-	+			
	Цитоплазма дей- томерита	-	+	+		+	+	+			
	Карноплазма	-	+	+		+	+	+			
	Нуклеолы	±	+	+		-	-	-			
15 Сформи- рованный тро- фозоит	Цитоплазма эн- мерита	-	+	±		±	+		-		
	Цитоплазма про- томерита	-	+	+	+	+	+	+	+		
	Цитоплазма дей- томерита	-	+	+	+	+	+	+	+		
	Карноплазма	-	+	+	+	+	+	+	+		+
	Нуклеолы	±	+	+	+	-	-	-	-		+
16 Гамонт	Цитоплазма про- томерита	-	±	+	+	+	+	±	+		
	Цитоплазма дей- томерита	-	±	+	+	+	+	±	+		
	Карноплазма	-	±	+	+	+	+	±	+		+
	Нуклеолы	±	+	+	+	-	-	-	-		+
17 Гамонто- циста	Цитоплазма	-	±	±		+	+	±			
	Ядро	+	+	+		+	+	±			
	Нуклеолы	-	+	+		-	-	-			
18 Ооциста	Цитоплазма	-	±	+		±					-
	Ядро	+	-	+		-					+
	Оболочка	-	-	-		-					-

- 1) Stage and Structure
- 2) Substance
- 3) DNA
- 4) RNA
- 5) Proteins
- 6) Histones
- 7) Paraglycogen
- 8) Acid mucopolysaccharides
- 9) Neutral fats
- 10) Lipids
- 11) Compounds of the phospholipid type
- 12)
- 13) Sporozoite: cytoplasm
nucleus

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Methods of Investigation

The Application of Oxyquinoline to the Study of Chromosomes

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In recent years a number of works has appeared in literature which recommend the use of oxyquinoline as a substance for demonstrating certain morphological structures of chromosomes, such as the centromeric areas, the secondary constrictions, etc. (Tjio and Levan, 1950; Sharma and Ghosh, 1950, and others). It is recommended that oxyquinoline be used either for the preliminary treatment of the biological object before fixation or as a constituent part of the fixative.

In the former case the biological object is placed in 0.002 M solution of oxyquinoline for three hours at a temperature of 10-14° C, and after a brief irrigation in water is fixed with some fixative. This method is used for preparing temporary preparations by the method of squashing with carmine stain, orcein or by the Feulgen method (Sharma and Ghosh, 1951; Sharma and Shattachardjee, 1952). In the latter case, 0.002 M oxyquinoline solution is added to the fixative fluid in a proportion of one part of oxyquinoline to three parts of one percent chromic acid and six parts of 10 percent formalin (Scharma and Ghosh, 1950, 1951). This method is used for the preparation of permanent preparations with embedment in paraffin. The advantages of the former method consists in an arrangement of

the
/ chromosomes more favorable for investigation.

In the work of Scharma (1956) it is mentioned that oxyquinoline increases the viscosity of the cytoplasm, which also leads to a more uniform distribution of the chromosomes throughout the cell. Afterwards, "the two limbs of the chromosome undergo compression from opposite ends ...", which leads to a demonstration of the centromere (Ibid., page 680).

For the purpose of elucidating the effect of oxyquinoline we compared wheat (Triticum monococcum) and barley (Hordeum nutans) chromosomes which had been fixed according to the Navashin method without preliminary treatment and with preliminary treatment using 0.002 M oxyquinoline solution for three hours. (Preparation of the oxyquinoline solution was as follows: 0.058 grams of oxyquinoline (8-oxyquinoline) is dissolved in 200 cubic centimeters of warm distilled water). The chromosomes from the periblem cells of the root tips were compared at more or less equal distances from the end of the root. The measurements, which were made with the same magnification, showed that under the influence of oxyquinoline the thickness of the chromosomes is increased by approximately 1.5 times whereas the length of the chromosomes remains practically unchanged (Fig., a-d). Sometimes the chromosomes are scattered throughout the cell so that the impression is created that there was a mechanical interference (Fig., d). The primary and secondary chromosome constrictions are very prominent.

The morphological structure of the chromosomes is even more prominent under the influence of oxyquinoline following a preliminary chilling of the material



Metaphasic plates of barley (on the left) and of wheat (on the right) after fixation according to the Navashin method.

- a, b) control;
- c, d) treated with oxyquinoline before fixation;
- e, f) chilled at 0°C with subsequent oxyquinoline treatment before fixation.

The Importance of the Internal Resistance of a Stimulator in the Investigation
of Nerve Excitability Under Conditions Where the Distribution of the Current in the

Stimulation Circuit Changes

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In our previous work (Vereninov, 1959) it was shown that with the alteration of nerve by certain substances the stimulation threshold is changed differently depending on the internal resistance of the stimulator. Thus, if the threshold was determined by means of a stimulator with a low internal resistance the excitability of the nerve altered with distilled water proved to be reduced; if a stimulator was used which had a high internal resistance the change in the excitability was diphasic, and during the first 20-40 minutes an increase in excitability was observed. A marked dependence of the ^{magnitude} and direction of the changes in excitability on the degree of internal resistance of the stimulator used in the experiment was observed also with the effect of acid, alkali, hypertonic solutions of sodium chloride and sucrose on the nerve.

Different threshold changes from the use of stimulators which differed only with respect to their internal resistance attested to a change in the distribution of the current between the excitable and parasitic elements of the stimulation circuit during the course of alteration of the nerve. In Fig. 1 a diagram is presented of such a circuit consisting of a stimulator -- source of

EMF (E) with a certain internal resistance R and a load in the form of electrode resistance r_e and nerve resistance. (In this work an ordinary capacitor chronaximeter with a 100 ohm shunt was used (Nasonov and Rozental', 1953).

In this case the voltage at the capacitor plays the part of the EMF and serves in practice as a measure of the excitability). In the simplest case the nerve should be regarded as a parallel hook-up of two resistors: the excitable element,

- the nerve fiber -- r and the resistance of the film of fluid, connective-tissue membranes and nerve fibers shunting them, for which the current remains subthreshold -- r_s .

According to the Kirchhoff law, the EMF acting in the stimulation circuit is distributed between the serially connected internal resistor of the stimulator, electrode resistor and nerve resistor (determinable by r_e and r).

The change in the resistance of any branch of the circuit during the experiment leads to a change in the coefficient of proportionality between the voltage drop in the nerve and the EMF which is recorded as the threshold for stimulation. With some interrelationships between R , r_e , r and r_s this proportionality coefficient depends very little on the changes in resistance of various branches of the stimulation circuit. Thus, if the internal resistance of the stimulator is much less than the total resistance of the electrodes and the nerve and the resistance of the electrodes is small by comparison with the nerve resistance almost the entire voltage of the oscillator falls on the nerve, and the threshold value E -- the measure of excitability -- does not depend on variations in the electrode resistance or in the parasitic shunt. Where the internal resistance of the stimulator is great by comparison with the resistance of the nerve and of the electrodes the measure of excitability does not depend on the electrode resistance; on the other hand it depends to a great extent on the degree

of shunting of the nerve fibers.

The analysis which was made (a more detailed presentation was given in our previous article) makes it possible to point out the cause of the different changes in the threshold value of E detectable in the same preparation with the use of stimulators having different internal resistances. It is clear, however, that in order to determine how the nerve excitability changes under conditions where there is a changing distribution of the current in the stimulation circuit it is necessary to evaluate, at least approximately, the magnitudes of electrode and nerve resistance and their change during alteration. The present work was devoted to this problem.

Methods

Measurement of the resistance of a nerve moistened with Ringer's or some other alternative solution and of the resistance of the electrodes was made by the method of oscillographic recording of the voltage in them during the passage of a rectangular pulsating current of a given amplitude. In accordance with the aim of the work methodological conditions of an ordinary alternative experiment were reproduced. The sciatic nerve of a frog was fastened horizontally to two isolated extenders located at a distance of 30-50 millimeters from each other. Two pairs of silver non-chlorinated (as in the previous experiment) electrodes 0.5 millimeter in thickness were brought up under the middle portion of the nerve. Two of them -- "current electrodes" -- were separated by 10 millimeters, connected to the square-pulse generator. The output impedance of which amounted to ≈ 11 milliohms. The two other electrodes -- "the measuring electrodes" -- were located as near as possible to the current electrodes, but were not in direct contact with them, but rather were hooked up to a differential amplifier and served for measuring the voltage in the nerve (Fig.2). (The

G.A. Mozhayeva amplifier, described in the work of G.N. Mozhayeva (1958) was used). By connecting one point of entry of the circuit into the amplifier with the measuring electrodes and the other with the current electrodes (lead-off between points two and three, Fig.2) it is possible to determine the magnitude of the voltage drop at the contact of the electrodes -- the droplet of fluid in the area of contact of the nerve with the electrode. For the purpose of controlling the shape of the current impulse passing through the nerve and the electrode the voltage was recorded in a two kilohm resistor which was hooked up in series with the electrodes (leads three-four). The resistance of the nerve and that of the electrode could be computed readily by the ratio of the voltage values in them and in the two kilohm resistor. The amplitude of the current impulse amounted, usually, to 0.5-3 microamperes, which corresponds to subthreshold or arathreshold stimulation strengths.

Typical oscillograms are presented in Fig.3. The voltage in the nerve, just as in the electrode, increases gradually from the effect of the direct current. In the nerve the voltage reaches a constant value after one to two milliseconds, whereas in the electrode it continues to increase up to the very end of the pulse (four milliseconds). The cause of this phenomenon, as is well known, consists of the electrochemical polarization of electrodes and polarization of the nerve. Such an increase in the voltage with a direct current corresponds to an increase in the resistance. Afterwards, we will deal only with the maximum value of the resistance determined at the end of the pulse.

The resistance of the nerve and the resistance of the nerve-electrode contact was measured during alteration of the preparation with 0.02 N HCl, five percent NaCl, 1 M sucrose and distilled water -- agents the influence of which on

the threshold magnitude, according to the data of the previous work, depended most strongly on the internal resistance of the stimulator. For the purpose of alteration the nerve and the electrodes were placed in a bath with the various solutions, and at the time of measurement they were lifted into the air.

Results

Even after the first immersion of the nerve and electrodes into the alternative solution (0.02 N HCl, five percent NaCl, 1 M sucrose, distilled water), that is, two or three minutes later their resistance changes markedly. Then, it remains almost at the same level until the end of the experiment (40-60 minutes). In Table 1 the ~~arithmetic mean~~ values are presented for the nerve resistance and for the resistance of the nerve-electrode contact obtained by moistening the nerves with Ringer's solution (25 experiments) and solutions of alternatives (six to seven experiments in each series). (The thickness of the nerves with which we worked was 0.5-0.6 millimeters).

As is evidenced by the data presented, after moistening with NaCl and HCl solutions the resistance of the nerve decreases by more than three times in the first case and by 1.5 times in the latter case. After moistening with distilled water and 1 M sucrose solution, on the other hand, it increases markedly.

A rapid change in the resistance of the nerve was produced primarily by a change in the resistance of the film of the fluid wetting the nerve. If we assume that distilled water practically does not shunt the nerve fibers, and this is very probable, "pure" resistance of the nerve (r) has a magnitude close

to 170 kilohms. Knowing the total resistance of the nerve fibers of the parasitic shunt and assuming that the resistance of the nerve cannot change rapidly and remains close to 170 kilohms for a certain time the resistance of the shunt (r_s) may be computed when the nerve is moistened with the solutions under investigation. The calculation shows that when the nerve is moistened with Ringer's solution only about 40 percent of the current passing through the electrodes goes through the nerve fibers and is effective in the sense of stimulation. When the preparation is moistened with five percent NaCl solution "the stimulating" fraction of the current is reduced to six percent, and when moistened with 0.02 N HCl, to 25 percent. Hence, it follows that with a varying shunt of the nerve it is impossible to evaluate the excitability according to the threshold strength of the current passing through the electrodes and, therefore, it is impractical to use a stimulator with a high internal resistance and a "stimulation strength" scale graduated in units of current strength.

The measurements showed that the resistance of the electrodes in contact with the nerve is of the order of two kilohms. It should be noted that polarization of the electrodes depends to a very great degree on the current density, that is, on the size of the drop at the electrodes; both may change markedly during the course of the experiment. Therefore, the values found should be regarded only as tentative. However, if it is assumed that the resistance of the electrode is even two to three times greater than was found in the present work it is still substantially less than the resistance of the nerve section 10 millimeters in length.

Since the resistance of the electrodes is small the EMF acting in the stimulation circuit, with the use of a stimulator having a small internal resistance, is applied to the nerve. The possible variations in the resistance of the electrodes and the resistance of the parasitic shunt cannot be expressed with the use of such a stimulator at the magnitudes of the threshold voltage. What has been stated means that with the use of a chronaximeter with an internal resistance of 100 ohms practically all the voltage at the capacitor plates (specifically this magnitude is recorded as "the strength" of stimulation) is applied to the nerve with any changes in the electrode resistance, shunt resistance or even resistance of the nerve fibers themselves which are possible in practice. Therefore, in this case the change in excitability can be judged by the change in the threshold voltage at the capacitor.

Therefore, for the purpose of determining the excitability of the nerve a stimulator should be selected with an internal resistance which is small by comparison with the resistance of the area being stimulated. For these reasons it is sometimes advantageous to increase the interelectrode distance.

How small the internal resistance should be depends on the accuracy of excitability determination required. Thus, for example, if the actual voltage in the nerve should not differ from the voltage at the capacitor by more than five percent the resistance of the stimulator should not exceed one kilohm ($60 \times 0.05 = 2$) with a nerve resistance of 60 kilohms and an electrode resistance of two kilohms.

Let us compare the results obtained with the data concerning the change

in the threshold with various alterations studied in the previous work. From what has been presented it follows primarily that the magnitudes of threshold changes obtained by means of a chronaximeter with an internal resistance of 100 ohms reflect the actual changes in the threshold which have not been distorted by a redistribution of the testing current because of changes in the nerve shunting. With a determination of the excitability using a chronaximeter having an internal resistance of 100 kilohms results were obtained which are too low after alteration of the nerve with solutions of 0.02N HCl and five percent NaCl and are too high after alteration with sucrose and distilled water. Knowing the "pure" resistance of the nerve and the resistance of the nerve-electrode contact after moistening the preparation with various solutions the difference of the threshold voltages obtainable through the use of the first and second chronaximeter variants can be computed for every case. According to this calculation the threshold voltage with the use of a stimulator having an internal resistance of 100 kilohms should differ from the threshold voltage obtained with an internal resistance of the stimulator of 100 ohms if the nerve is moistened with Ringer's solution by 2.5 times; with distilled water, by 1.6 times; with NaCl solution, by 6-7 times; with HCl by 3.4 times; and with sucrose, by 1.8 times. In the previous work the difference in the threshold amounted, respectively, to 3.3; 1.4; 7; 5 and 1.5 times. As a first approximation such a coincidence of the results may be considered satisfactory. (The measurements made convince us that the usual determinations of the excitation threshold without oscillographic control of the stimulation strength and of the response reaction, as occurred in the previous

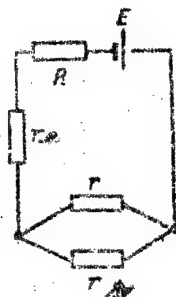


Fig. 1. Diagram of Stimulation circuit.
Explanations in text.

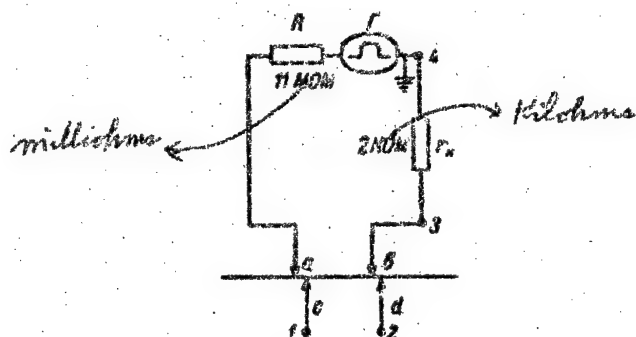


Fig. 2. Diagram of circuit for measuring resistance of nerve and nerve contact -- the electrode.
R) internal resistance of stimulator (1), r_c) calibrated resistor; a, b) "current electrodes"; c, d) measuring electrodes. The figures designate the individual points in the circuit. The rest of the explanation is in the text.

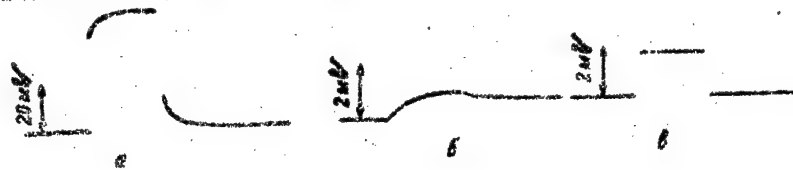


Fig. 3. Oscillograms of voltage changes.

a) in the nerve; b) in the area of contact between the nerve and electrode; c) in the calibrated 2-kilohm resistor under conditions where the preparation is moistened with Ringer's solution. The duration of the pulse is four milliseconds.

Table 1

Average Values for Nerve Resistance of Nerve-Electrode Contact After Moistening the Nerve with Various Solutions

① Сопротивление (в кОм)	② раствор Рингера	③ 5% NaCl	④ 0.02 N HCl в растворе Рингера	⑤ 1 M сахара в растворе Рингера	⑥ дистиллированная вода
⑧ Нерв (участок 10 мм) M ± m	61 ± 0.06	17 ± 1.25	40 ± 0.4	111 ± 5.9	162 ± 4
⑨ Контакт нерва-электрод (M ± m)	1.4 ± 0.1	0.53 ± 0.04	0.85 ± 0.05	1.96 ± 0.16	5.2 ± 0.17

- 1) Resistance (in kilohms);
- 2) Ringer's solution;
- 3) 5% NaCl;
- 4) Solutions;
- 5) 0.02 N HCl in Ringer's solution;
- 6) 1 M sucrose in Ringer's solution
- 7) Distilled water
- 8) Nerve (10 millimeter section) M ± m
- 9) Nerve-electrode contact (M ± m)

work, are too rough, so that better agreement may be required). It is perfectly obvious that the change in the coefficient of proportionality between the voltage ~~and~~ at the capacitor and the voltage in the nerve (see Table 2) after the application of the alterative solution produces changes in the curve of threshold changes during testing with a stimulator having a high internal resistance. It is specifically in this manner that the picture of a primary reduction in the excitability occurs after the effect of solutions of NaCl and HCl on the nerve and an increase in excitability under the influence of distilled water and sucrose. On the basis of what has been stated, these changes should be considered artefacts.

Table 2

in

Voltage in the Nerve/Percent of EMF of Stimulator (Voltage at the Capacitor) With
an Internal Resistance of 100 Kilohms After Various Alterations

Ringer's solution	Solutions			
	5% NaCl	0.02 N HCl	1 M Sucrose	Distilled water
38	14.5	28.6	52	62

The data obtained in this and the previous work remind us of the necessity for careful analysis of the causes of ^{stimulation} threshold changes. The magnitude of the latter is determined by many factors, including also "physical", and therefore cannot serve as a direct measurement of excitability.

Resume

1. The values for the resistance of the nerve and the nerve-electrode contact were determined after the preparation was moistened with Ringer's solution and after alteration of the nerve with 0.02 N HCl, five percent NaCl,

distilled water and one M sucrose.

2. An explanation has been proposed for the dependence of the magnitude of threshold changes on the internal resistance of the stimulator after alteration of the nerve.

3. The data obtained permit us to recommend the use of stimulators having a low internal resistance (no more than one to two kilohms) for the determination of nerve excitability after the effect of alterative solutions.

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Measurement of the Thickness of Ultrathin Sections by the

Calculation Method

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In the study of ultrathin sections in electron microscopy the thickness of them is of great importance (Gettner and Ornstein, 1956; Borysko, 1956, and others). Ultramicrotomes serving for the purpose of obtaining sections usually have adjustable scales which only indirectly show the thickness of the sections being obtained. Nevertheless, the measurement of them is associated with certain difficulties. From the literature available to us several methods of determining the thickness of ultrathin sections may be described.

1) Method of the light wedge (Frimer, 1956) is based on the reflection of a fine monochromatic beam of light from two translucent mirrors applied to each other and between which the section has been placed. By the interference picture the angle of divergence of the mirrors and the thickness of the section are determined. The measured section cannot afterwards be used for examination.

2) Method of elipsometry (Faucher, McManus and Trurnit, 1958) was based on the principle of multiple internal reflection of a polarized monochromatic beam of light on a film.

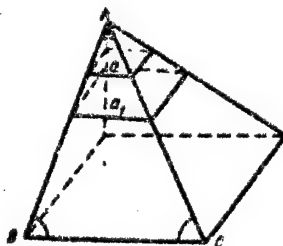
3) Method of colored scale (Peachey, 1958) is based on the change in

the hue of a section with a change in its thickness. It makes it possible to determine a thickness of the section no smaller than 60 millimicrons.

These methods of measuring the thickness of sections are complex, require the application of special devices or give only approximate results.

We have made use of methacrylate blocks (butyl-methyl methacrylate 8:1) with a 1.5 percent benzoyl peroxide catalyst polymerized in an incubator at 45-50° for 12-18 hours (Newman, Borysko and Swerdlow, 1949).

The sections were prepared on a Sjöstrand ultramicrotome and a rocking microtome (Cambridge model) with glass knives prepared by method utilized in a number of investigations (Latta and Hartmann, 1950; Cameron, 1956; Borovyagin, Yefimov and Dubrov, 1958). The methacrylate block was given the shape of a pyramid one of the planes of which was sectioned perpendicularly to the base, as is represented in the Figure. With the obtaining of the section the height of the pyramid was reduced. Measuring the width of the front border of the first and last sections and knowing the two angles, we can compute the height of the sectioned portion. Dividing this height by the number of sections we can determine the average thickness of the sections, computing it according to the formula.



$$\text{average thickness of section } \left(\frac{a_1 - a}{n \cdot \sin A \cdot 10,000} \right) \sin B \cdot \sin C$$

The values a and a_1 are determined by measuring the front borders of the first and last sections with a micrometer ocular. The angles A , B and C are angles of a triangle, which is in a plane perpendicular to the base.

We measured them by means of a miniature protractor printed on a celluloid photographic film by the photographic method which was then placed in the micrometer ocular. Use may also be made of an ordinary school protractor, attaching it to the outside of the microscope tube and making it coincide with the ordinary micrometer ocular. A determination of the angle A is not obligatory, because it is equal to $180^\circ - (B/C)$. At the same time, measurement of this angle gives a good control of the accuracy of measurement of the angles, because $A+B+C = 180^\circ$.

The value of the sines of the angles obtained is determined by a table of trigonometric functions. The answer is given in ["]Angströms["].

The method being proposed gives quite a high degree of accuracy. The error, calculated according to ~~g~~ well known formulas without taking into consideration the compressibility of the sections (particularly since these deformations can be eliminated by treatment of the sections with xylol vapors) does not exceed three percent.

The error is reduced by increasing the number of sections. For greater accuracy the thickness of a series in which the number of sections is no less than 400 should be measured.

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Methods of Extraction from Fine Unfixed Sections for the Purpose of

Studying the Histochemistry of Cell Structures

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In the work of one of us (Georgiyev, 1958) a method of serial extractions
from thin unfixed sections was used for the purpose of cytological characterization
of protein and nucleoprotein fractions obtained from isolated cell nuclei

(Zbarskiy, 1950; Downs, 1957). It was shown by this method that the globulin
fraction, which is soluble in 0.14 M NaCl, consists of nuclear

sap, a nucleoprotein fraction extractable with 1 M NaCl, ^{and} represents the
entire desoxyribonucleoprotein of the chromatin; the fraction of acid protein
extractable by alkali represents the RNA and nucleolar proteins and "the residual
chromatin" ("residual chromatins"; Georgiyev, 1958). In subsequent works (Zbarskiy
and Georgiyev, 1959a, b) we studied the fractionation of nuclei

isolated under conditions which maximally preserve their natural state
by means of these solvents, and we determined the protein and nucleic-acid content
in the fractions isolated. The results obtained completely confirmed the
conclusions of the first investigation.

Therefore, a series of data was obtained on protein and nucleoprotein
composition of the intranuclear structures (Zbarskiy and Georgiyev, 1959a, b;
Georgiyev, 1959a).

Taking into consideration the fact that chemical fractionation of
isolated nuclei completely confirmed the results obtained by means of successive

extractions from unfixed sections we are presenting a detailed description of this method, and an analysis of its possibilities and ^{some} experimental data obtained by means of it which were not included in the articles mentioned.

Description of the Method

Methods of extraction from unfixed sections permit us to establish the localization of proteins or nucleoproteins in the cell structures on the basis of their solubility in various solvents and also to investigate certain aspects of the physico-chemical condition of proteins in cell structures. Since the solubility of these substances depends on various denaturing influences, the tissue should not be subjected fixation or embedding before extraction. Even lyophilization of the material leads to a change in the solubility of a number of proteins, particularly in the desoxyribonucleoprotein (Ambrose, 1956). Removal of the soluble component from the section in many cases depends on the absence of obstacles to the diffusion of ... molecules with high molecular weight, for example, those of the nuclear membrane or of the cytoplasmic layer adjacent to the nucleus (Georgiyev, 1958).

Our method is distinguished from the methods used by other authors who also worked with the extraction of tissue sections (Mirsky and Pollister, 1946; Panijel, 1951) by the observance of these conditions. The experimental data which we obtained to a considerable degree do not agree with the results of these works in which lack of observance of these conditions led to a distortion of the extraction results.

For the purpose of preparing thin unfixed sections use was made of a

freezing microtome with a deep cooling knife (Romeis, 1954, page 187). Since there was no special microtome supplying carbon dioxide to the knife at our disposal we used a simple adaptation for cooling the knife. It consists of four copper tubes which during operation are inserted into a hole into the table serving for the administration of carbon dioxide. The tubes are bent in such a way that they direct the carbon dioxide to the lower surface of the knife along its entire length. The carbon dioxide coming from the other holes is directed toward the table by means of a bell jar, where it precipitates in the form of a snow. In order that the knife remain chilled for quite a long time the precipitated carbon dioxide is collected and applied to the knife. Good sections are obtained if the tissue block is not overfrozen and the knife is cooled sufficiently.

For the purpose of obtaining thin sections (from three to four microns) an important condition is the good quality of the knife. With a proper interrelationship of the knife temperature and the temperature of the tissue block the sections do not crumble and do not thaw out. They either separate out in a smooth condition, or they twist into a tube. The section should not be brought to the end. If the section is twisted it is carefully straightened out on the surface of ^{the} knife with a dry brush. Afterwards a dry defatted glass slide is applied to the section; on contact with it the section immediately straightens out completely and adheres to it. In the majority of our experiments the glass slides were preliminarily chilled to 2-4°. Attachment to unchilled glass slides occurs even better. After attachment ^{to} the glass slide the sections

are immersed in an extractive solution, as quickly as possible to avoid desiccation, ^{and} chilled to 0-2°. Part of the sections which are the controls are fixed immediately.

After extraction for various intervals of time the sections are fixed and stained like the control sections. In almost all our experiments absolute alcohol served as the fixative (treatment for two minutes). Then, the section was passed through 96 percent and 70 percent alcohol and water for one or two minutes each before staining. Under these conditions, the thin cytoplasmic structure of the tissues was preserved, and the sections practically never became loose during the subsequent staining for proteins and nucleic acids (bromphenol blue, pyronine-methylgreen, Feulgen reaction). In some experiments other methods of fixation were used (formalin, OsO₄ vapors), whereby even under these conditions completely preserved preparations were obtained.

After extraction with salt solutions of different ionic strength in a neutral medium these sections remained well on the glass slide, and the extraction could be carried out with the glass slides in a vertical position in the container. After distilled water extraction or extraction with alkali solutions a removal of the main mass of proteins of the sections occurs, as a result of which the matter easily loses its adherence. To avoid this the treatment should be carried out in Petri dishes with the glass slide containing the section in a horizontal position, aspirating the solvent at the end of the extraction and pouring in fixative in place of it.

In the procedure described, freezing of the material from the very beginning of the experiment and the utilization of chilled solutions also contributes, in addition to the elimination of fixation before extraction, to the preservation of the natural state of the proteins (absence of denaturation and

autolysis). A direct transfer of the section from the knife to a glass slide without straightening it out in distilled water eliminates a loss of proteins, which is inevitable ^{after} contact with the water.

Therefore, the method described permits us to study in its purest form the solubility of proteins and nucleoproteins of cell structure.

Since the solubility in various solutions is characteristic of the given protein component and, particularly, indicates the natural state of the latter, the method described can be used not only for cytological characterization of protein fractions of the nucleus but also in the study of cytoplasmic structures, for the characterization of the state of various proteins in the cell under various conditions, the effect of injurious agents, in the histochemistry of enzymes, etc.

Study of Nuclei of Prenecrotic Tumor Cells

One of the examples of application of the method ^{is} presented below. As we have already noted, 1 M NaCl rapidly extracts the entire desoxyribonucleoprotein of the cell nucleus, leaving the RNA ^{and} the proteins of the nucleoli ^{of} and/the "residual chromatin".

In the study of sections of a subcutaneous mouse Ehrlich carcinoma the foci of necrosis filled with detritus were not stained in staining for nucleic acids. The border between necrosis and viable tissue is always very sharply expressed. It consists of one to three rows of cells, the nuclei of which are stained more intensely both by the Feulgen method and with methylgreen, probably by virtue of the pycnotic changes (Fig.1). The intensity of their staining is similar to the intensity of staining of mitotic chromosomes. Usually the cells of this transitional layer are without nucleoli, but sometimes they contain quite large nucleoli stained with pyronine (Fig.2).

The desoxyribonucleoprotein of the nuclei of the transitional layer is practically not extracted with one M NaCl and maintains the capacity for being stained by both methylgreen and by the Feulgen method (Fig.2, 3). No effect is exerted on the nuclei of the prenecrotic layer by 0.4 M NaCl, which produces a "homogenization" (washing out) of the nuclear desoxyribonucleoprotein of the viable cells (Georgiyev, 1959b), even though it does not extract.

Therefore, the prenecrotic cells are different from normal cells primarily in the physico-chemical changes of the desoxyribonucleoprotein complex, manifested in a loss of solubility in one M NaCl, rather than in a loss of the nucleolar apparatus or degradation and denaturation of the DNA (staining with methylgreen is maintained!). The changes apparently occur very rapidly, because such cells make up no more than two or three rows. The decomposition of DNA and RNA occurs at a later stage.

Discussion of Results

As seen from the present and preceding reports, the method of extraction from thin unfixed sections makes it possible to obtain a series of valuable cytochemical and histochemical data. However, the following limitations of the method should be kept in mind.

1) During freezing the formation and growth of ice crystals occurs which may lead to dehydration of the object and injury of the structures.

2) Certain structures markedly change their permeability (for example, mitochondria, which thereby liberate a number of enzymes; Downs, 1957) during freezing and thawing.

3) The possibility of removal of soluble components at the time of freezing and thawing has not been excluded.

In our experiments on the localization of nuclear fractions these factors could hardly have any significant influence on the results obtained.

The cytological picture in our preparations was distinctly expressed, and the number of injured cells was small. The nuclei did not undergo any noticeable changes from the preliminary freezing, which follows from an analysis of the series of works for isolation of them. The harmful effect of the latter is apparently expressed only in the liberation of mitochondrial nuclease with subsequent thawing (Downs, 1957). However, with extraction from sections dilution of the enzymes with the extracting fluid is very great. The probability of enzymic effect is reduced to an even greater degree with extraction by 1 M NaCl because of the inhibitory effect of the latter on

both desoxyribonucleases. Finally, the removal of the desoxyribonucleoprotein material of the chromosomes of the nucleolar material apparently did not occur, because otherwise we would readily have detected it histochemically. The possibility of a certain displacement of the globulin fraction has not been entirely excluded, but even this could not distort the principal results, because at least part of it is preserved.

Therefore, the limitations of the method presented in our experiments could hardly have had any effect on the results, particularly since the latter were clear-cut and the same in almost all experiments. As far as the application of the method is concerned for solving other problems, a strict account of these considerations should be made in every specific case.

Apart from the applicability to the study of the protein and nucleoprotein composition of cellular structures some of the procedures used may also be applied in ordinary laboratory practice. This is primarily the preparation of sections in a freezing microtome by means of a simple device which chills the knife. In preserving a satisfactory histological and cytological picture the method makes it possible to obtain the finished preparations very quickly. Thus, we obtained sections stained with pyronine-methylgreen and ready for study as early as 30-40 minutes after the animal was killed (cutting--10 minutes; fixation and passing through solutions up to water -- five minutes; staining -- 10-20 minutes; passage and embedding in Canada balsam -- five minutes). With other methods of staining the finished preparation can be obtained every more quickly.

We readily obtained sections with a thickness of from three-four to eight-15 microns from the following organs: liver, pancreas, intestine, kidneys, spleen, spinal cord, spinal ganglia, skin, muscles, hepatoma and mouse Ehrlich carcinoma, rat liver, liver, suprarenal glands and the total preparation of a rat embryo, liver and spinal cord of ^a guinea pig and rabbit, intestine and heart of a rabbit.

Therefore, with just a little practice it is possible to obtain the sections from practically any tissue and to ^{make} stained preparations from them quickly. On this basis the method may be recommended for emergency biopsies in pathology laboratories. At the present time, the fixation of material with hot formalin and subsequent preparation of the sections in a freezing ^{microtome} by the usual method has become widespread. Here, roughness in fixation leads to marked distortions of the histological picture. The use of a deep cooling knife makes it possible to make preparations more rapidly and to obtain a better preserved histological picture, since fixation of a section may be accomplished ^{by} much gentler method and in a shorter period of time than fixation of a small chunk of tissue.

Conclusions

The technique has been described ^{and the} /possibilities and limitations of an extraction method for thin (3-4 micron) unfixed sections prepared in a freezing ^{microtome}, by means of a deep cooling knife have been analyzed. The method ^{was} applied to the study of preneoplastic cells of a subcutaneous Ehrlich carcinoma. The desoxyribonucleoprotein of the nuclei of these cells loses its solubility in 1 M NaCl. This change precedes the

degradation of DNA and the loss of nucleoli.

It is recommended that preparations be obtained from unfixed tissue in a freezing microtome by means of a deep cooling knife for emergency biopsies. A simple adaptation is described for chilling the knife.

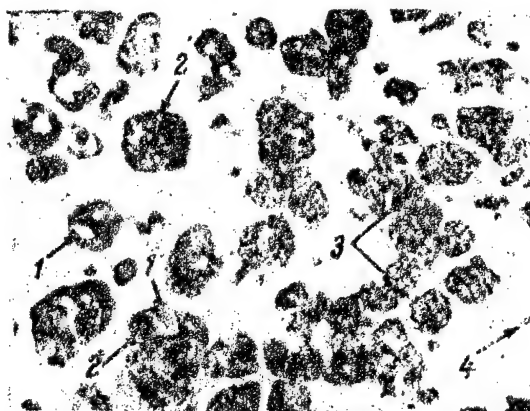


Fig. 1. Section (3-4 microns) of Ehrlich Carcinoma staining by the Feulgen method; magnification, 100 x 10).

1) nucleoli (Feulgen-negative); 2) perinuclear chromatin; 3) pycnotic cells of preneurotic layer; 4) area of necrosis.



Fig. 2. Section (3-4 microns) of Ehrlich carcinoma following extraction with 1 M NaCl for 18 hours (staining with pyronine-methylgreen, magnification 40 x 10).

1) area of viable tissue (desoxyribonucleoprotein removed);

- 2) nucleoli;
- 3) nuclei of cells in area of necrosis devoid of nucleic acids;
- 4) nuclei of preneoplastic layer preserving the desoxyribonucleoprotein;
- 5) nucleoli in the nuclei of the preneoplastic layer.



Fig. 3. Section (3-4 microns) of Ehrlich carcinoma after extraction with 1 M NaCl for 2 hours (staining by the Feulgen method, magnification 40 x 10).

- 1) area of viable tissue from which the desoxyribonucleoprotein has been removed by extraction;
- 2) nuclei devoid of DNA in necrotic area;
- 3) filament of desoxyribonucleoprotein extracted from the nuclei, precipitated with alcohol;
- 4) nuclei of cells of preneoplastic area preserving their desoxyribonucleoprotein.

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Critique

By D. N. Nasonov, "Mestnaya reaktsiya protoplazmy i rasprostranyayushcheyesya vozbuzhdeniye", (Local Reaction of Protoplasm and Spreading Excitation). Published by the Academy of Sciences USSR, Moscow - Leningrad, 1959, 434 pages, 218 figures.

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This book by corresponding member of the Academy of Sciences USSR Professor Dmitriy Nikolayevich Nasonov represents an outstanding achievement in Soviet scientific literature. It is devoted to a cardinal topic of general physiology -- excitability and excitation. This topic has been given a new approach by the author. The richest experimental material obtained by Nasonov and his co-workers and a profound critical analysis of the data in the literature which is given in an illustrious manner on the pages of the book show the ~~worthlessness~~ of a number of the basic conceptions existing in modern general physiology. In place of them the author has proposed an entire system of organically related new concepts which make it possible to understand better the existing facts and to outline a new means for studying the nature of excitation. The author's main attack was directed at the membrane theory of permeability and excitation, the essential defects of which are disclosed by the author with irresistible argumentation. Based on numerous facts and interesting considerations, D.N. Nasonov proposes the sorption theory of permeability and the denaturation theory of excitation.

The first part of the book is devoted to an investigation of the local reaction of living protoplasm to environmental influences. Here, a

presentation is given of the well-known theory of paranecrosis of D.N. Nasonov and V.Ya. Aleksandrov, and problems are discussed concerning the interrelationship of the local reaction and excitation. Tremendous factual material makes it possible for the author to identify the condition of excitation, injury and narcosis: "...we are inclined to regard muscle narcosis produced not only by typical narcotics but also by ^{non-specific} stimuli as a refractory nature of a non-varying ^{standing} excitation, manifesting itself outwardly in a contracture" (page 70). The idea of a standing non-varying local excitation, which was developed by N.Ye. Vvedenskiy in his theory of parabiosis, has found a new solid confirmation in the investigations of Nasonov and his school.

The second portion of the book is devoted to problems of excitation and permeability, which are most closely connected with each other. In this part a criticism is given not only of the membrane theory but numerous facts are also presented on behalf of the sorption theory of permeability. The author addresses very weighty remarks to the current "theory of selective ^{pumping-out} mechanisms" (Rosenberg, Wildbrant, Lundegard, Krogh, Hodgkin and others). The following statement by Nasonov is essential: "...the sorption theory asserts that the existing distribution of substances may be created and maintained only under conditions where there is an energy consumption through metabolism. In this respect there are no differences between the requirements of the two theories (the theory of the pump and the sorption theory -- Ye.Zh.). The difference consists only in the opinion of the nature of the forces which in the practice accomplish the distribution and redistribution of the substances.

The pump theory does not give any specific answer to this question. As far as the sorption theory is concerned, it gives a very definite answer concerning the distribution factors. These factors are the following: the solubility of the substance in the protoplasmic water, absorption on micellar surfaces of protoplasmic colloids, and the chemical bond with the substrate. All three factors are variable and depend, to a great degree, on the physiological state of the protoplasm. This also accounts for the level and the nature of distribution of substances between the cell and the medium" (page 177).

The third part is devoted to an analysis of the bioelectrical potentials. The author formulates the phase theory of bioelectrical potentials and gives interesting proof on its behalf. "From the viewpoint of the phase theory of bioelectrical potentials being developed the direct cause of the occurrence of electromotive force at the surface of the cell is a difference in the ion concentrations in the two areas of protoplasms; however, this difference does not precede but rather appears suddenly during injury or stimulation because of a degeneration of an unstable chemical compound -- the salt-protein complex. The cause of this degeneration should be considered a reversible denaturation of proteins in the initial stages" (page 211). Unfortunately, the solidly established facts concerning the presence of polarized structures in nerve and muscle fibers obtained by direct measurements of the capacitance and resistance of these structures were not properly analyzed in the book.

In the fourth part the book deals with spreading excitation and its relationship to the local reaction. The cleverness of experiments and

originality, the wisdom and convincing nature of the theoretical constructs of the author are a real pleasure to the reader. Without any doubt, the "step-wise theory of spreading excitation" and "the law of self-regulation of the spreading excitation wave" are a culmination of Nasonov's talent and one of the most outstanding achievements of general physiology of recent time. The author succeeds also in clarifying a confusing question concerning the decrement and absence of decrement in the spread of excitation and in clarifying the conditions under which these forms of spreading excitation occur.

The fifth and sixth parts are devoted to problems of excitometrics. In them D.N. Nasonov shows himself to be an accurate, scrupulous experimenter and an original thinker. He launches an avalanche of experimental and theoretical criticism of the conception of chronaxie which is still holding out in physiology. Sometimes, excessively bitter criticism has been of essential benefit for the clarification of our methods of measuring excitability. An interesting method of measuring "brief" and "long" excitability was proposed by Nasonov himself.

D.N. Nasonov's entire book is impregnated with a militant spirit of scientific criticism of the customary positions which are still held in general physiology, with the spirit of an innovator, and of creativeness. In the fire of strenuous research work new ideas are being forged which more correctly and more accurately reflect the truth. The book will constitute the idea for and will inspire new research. Herein is its particular scientific importance.

The book is very well edited, but its circulation (a total of 2000!) is completely inadequate: even now it represents a bibliographic rarity.

Ye. K. Zhukov

Annals

Lev Konstantinovich Lozina-Lozinskiy

(On his 60th birthday)



December 1959 marked the 60th birthday of Lev Konstantinovich Lozina-Lozinskiy, Doctor of Biological Sciences and Director of the Laboratory of cell adaptations of the Institute of Cytology of the Academy of Sciences USSR.

K. K. Lozina-Lozinskiy was born in 1899 in St. Petersburg into a physician's family. In 1918, on completing gymnasium [secondary school] with a gold medal, he was admitted to the geographic faculty of the University of Petrograd, from which he was graduated in 1924. A considerable part of K. K.'s scientific activity occurred in the Natural Scientific Institute imeni P. F. Leshaft, where he was admitted in 1922 while still a student, and where he worked (with minor interruptions associated with evacuation during the period of the second world war until 1955, starting as a preparer [equivalent to a laboratory technician] to director of the laboratory. In 1955, L. K. began to work in the Laboratory of Cytology of the Zoological Institute of the Academy of Sciences USSR at the invitation

USSR at the invitation of D.N. Nasonov, and then, beginning with 1957, and in connection with the reorganization of the laboratory in the Institute of Cytology, he became the director of the Laboratory of Cell Adaptations of the Institute. Along with his scientific work L.K. occupied himself also in pedagogical activity in college. From 1927 through 1935 he taught biology in the Institute of Physical Culture imeni P.F. Leshaft in Leningrad, and during the period of evacuation he gave a course at the University in Kazan'.

L.K. Lozina-Lozinskiy is a specialist in a broad field of biology. He has published more than 80 works in various fields of zoology, protistology and ecology, including a number of important investigations on the physiology of unicellular animals. Despite the variety of objects/which L.K. has worked the majority of his investigations is connected with the problem of adaptation of organisms, tissues and cells to existential conditions including to various extreme environmental factors (low temperatures, ultra-violet irradiation, the effect of ultrasound waves, etc.). L.K. has given considerable attention to the behavior of organisms as one of the forms of adaptation.

In/ ^a number of protistology ^{of} investigations/L.K. his works on the physiology of nutrition and behavior of protozoans has enjoyed wide renown. In his monograph "The Physiology of Nutrition of Infusorians" (1931) complex relationships are revealed which determine the nature of phagocytosis, its intensity depending on the physiological state of the infusorians, the nature of the food and the environmental factors.

The works of L.K. on soil protozoans are of great interest. He has

succeeded in working out an original method for studying the rapidity of the spread of protozoans in the soil and for proving indisputably their existence in the soil in an active state.

A series of works by L.K. is devoted to problems of insect ecology. In them observations are combined which were made in nature with accurately performed laboratory experiments. He has made a detailed study of the behavior of a number of important agricultural pests in nature (corn borer, boll-worm, etc.) and, particularly, has investigated in special detail the conditions determining the selectivity of oviposition. During the course of these ecological-physiological works on insects L.K. participated in a whole series of expeditions to the tropical areas of the Soviet Union (Azerbaijan, Bashkiria, Kalmytskaya Autonomous Oblast' and others). In the course of the ecological-physiological research in insects L.K. investigated resistance to cold in particular detail and with particular thoroughness and the conditions which determine it. He succeeded in showing the possibility of very deep-seated chilling of certain caterpillars (to a temperature to the order of $-70-0^{\circ}$) with subsequent recovery of the vital functions of the tissues. L.K. has devoted several works to problems of the influence of low temperatures and of freezing on the tissue of vertebrate animals and protozoans. L.K. Lozina-Lozinskiy is the greatest specialist in the Soviet Union in the area of studying phenomena of anabiosis in chilling. His works in this direction are acquiring particular interest at the present time in connection with the necessity for a detailed investigation of the conditions which will affect the organisms in cosmic space.

L.K. Lozina-Lozinskiy has published a number of purely zoological works on the classification and zoogeography of the pantopods, a distinct group of sea arthropods. For the purpose of collecting material on this group L.K. participated in several expeditions to the Arctic Seas. In this part of his work he was in close association with the well known Russian hydrobiologist, Professor Konstantin Mikhaylovich Daryugin.

L.K. has done a great deal also for propaganda and the popularization of biological knowledge, particularly among secondary school biology teachers. Under his editorship and with his participation the Academy of Pedagogical Sciences has published an interesting book for teachers -- "Invertebrate Animals". In this book L.K. has written an extensive and very interesting chapter on forms of adaptation of the body to environmental conditions.

In recent years at the Institute of Cytology L.K. Lozina-Lozinskiy has developed very interesting and promising work both for cytology and for medicine with respect to the study of the effect of ultra-violet rays on the cell. The object of these investigations consisted chiefly of the infusorians.

He and his co-workers are investigating conditions of cell repair following injury by ultra-violet rays, and have particularly shown the great importance of radiant energy in the visible portion of the spectrum in these processes. Visible light plays an important part in the reactivation of the cell following ultra-violet injury. Most recently, L.K. has posed the question, which is of great theoretical importance, concerning the possibility of cell adaptation to injurious doses of radiant energy and has worked it out experimentally.

During his many years of scientific work L.K. has ^{trained} a number of scientific workers from the group of graduate students, who worked under his direction.

A characteristic feature of L.K. as a person and as a scientist is his devotion to science. It would be hard to imagine him outside of his laboratory and outside of his fervent scientific activity. These qualities of L.K. ^{were} shown in particularly striking fashion during the organization of the Institute of Cytology. In a short time he created an actively working laboratory with thematics of great current importance.

It is ^{pleasant} : to note that . : L.K.

Lev Konstantinovich has come to his 60th birthday full of creative strength and broad scientific plans. He is continuing his experimental scientific work with great energy, and his creative enthusiasm is contagious to his students and his comrades in work. We should like to wish ^{creative} Lev Konstantinovich further great/achievements in his fruitful scientific activity as a Soviet scientist.

Yu. I. Polyanskiy

Coordination Conference on the Problem "Key Questions in Cytology"

By A.A. Vereninov, Yu.L. Goroshchenko and A.L. Yudin

The first coordination conference on cytology was a great event for those working in the field of cytology and sciences contiguous with it. As is well known, in recent years a number of measures have been undertaken designed to contribute to the growth of Soviet cytology. The Institute of Cytology in Leningrad, the Institute of Cytology and Genetics in Novosibirsk and the Institute of Radiation and Physico-Chemical Biology in Moscow were created; new cytological laboratories were opened up in a number of institutions. Beginning with 1959 the journal Tsitologiya has been published. Finally, investigations on cytology have been devoted to a particular problem by the directorate of the academy of sciences -- "Key Questions of Cytology" -- and a scientific council has been created on this problem. The conference held from 12 to 17 October in Leningrad is a natural development of this tendency.

The first session was devoted to a general survey of the most important trends in cytological research. A.S. Troshin, who spoke in the name of the scientific council (Institute of Cytology of the Academy of Sciences USSR, Leningrad) presented the principal tasks of research on the problem "Key Questions of Cytology". Among them, as has been mentioned, the question of the mechanisms of cell reproduction and of elementary cell structures is of the greatest importance. The investigation of this problem makes it possible to establish the causes of the transition of cells from a "resting" state to cell division, the role of nucleic acids in the synthesis of protein and the processes

of reaction between the nucleus and cytoplasm which is of great importance for solving a number of practical problems. On the basis of the achievements of modern experimental cytology investigations should be carried out on the nature of malignant growth -- a second very important current trend in cytological work. A study of the mechanism of maintenance of an asymmetrical distribution of substances between the cell and the medium in the presence of a continuous exchange was analyzed in a report as the third most important field of investigation. The condition of water and mineral substances in the cell, the physical structure of hyaloplasm, the role of the membrane and the part played by the enzymic reactions in the transportation of substances constitute the group of problems which needed to be worked out primarily. A study of irritability was considered an important trend both in connection with the analysis of a general non-specific basis of the cell reaction to external influences and with the study of the reparative and autoregulatory capacities of cells and along the line of studying the specific reactions and specialized functional acts of cells: contraction, the production of electrical energy, secretion, etc. In the division of the cytology of unicellular organisms the inherent necessity was noted for a comparative cytological study of bacteria, blue-green algae, spirochetes and actinomycetes from the viewpoint of the organization of their protoplast, an analysis of the characteristics of metabolism in connection with the shift of polyploid and oligoploid phases and the presence of filterable forms. Along with the divisions of cytology listed the beginning of a new, rapidly developing branch of cytology was noted -- cytoecology, which combines problems associated with the study of cellular

adaptations and their role in the adaptation of organisms to existential conditions.

The next report by M.N. Meyseľ' (Institute of Microbiology of the Academy of Medical Sciences USSR, Moscow) and A.A. Prokof'yeva-Bel'govskaya (Institute of Biophysics of the Academy of Sciences USSR, Moscow) was devoted to problems of radiation cytology. After noting the great practical importance of radiation cytology, M.N. Meyseľ distinguished the following research trends as the principal ones: the rules and regulations of the injurious effect of radiation on the nucleus and the cytoplasm, the mechanisms of injury of cellular functions, the mechanisms of the action of protective substances, the nature of radioresistance and the possibility of adaptation to the effect of radiation, the nature of the malignifying effect of radiation, and radiation as a factor in directed variation in heredity. The speaker emphasized the need for studying the rules and regulations of cell reactions as a whole from the effect of radiation. In his opinion, the further development of radiochemical problems, particularly for such complex structured heterogeneous systems as protoplasm, is of great importance.

In the second portion of the report A.A. Prokof'yeva-Bel'govskaya demonstrated the various types of injury to the chromosomal apparatus from the effect of radiation. The significance of radiation injuries to the nucleus very was considered/important in connection with the problem of the genetic effect of radiation. The speaker directed attention to the fact that primates, including man, apparently possess a particularly high degree of radiosensitivity, which is evidenced by data obtained recently by G.G. Tinyakov and M.A. Arsen'yeva on monkeys.

G.M. Frank (Institute of Biophysics of the Academy of Sciences USSR, Moscow) spoke of the ^{routes} of investigation of the submicroscopic structure of cells and tissues and pointed out, particularly, the need for a more extensive incorporation of electromicroscopy into research practice. Using the material of work accomplished chiefly at the Institute of Biophysics, the speaker demonstrated the possibility of studying the "molecular anatomy" of protoplasm.

The evening session on 12 October was devoted to problems of cytoecology.

V.Ya. Aleksandrov (Botanical Institute imeni V.L. Komarov of the Academy of Sciences USSR, Leningrad) formulated the problem of cytoecology as a science of adaptive reactions which are accomplished at the cell level, and noted the importance of this trend in work for solving problems of acclimatization, cold-, heat-, drought- and salt-resistance of plant and animal organisms and the analysis of the phenomenon of cell resistance to the effect of antibiotics, bactericidal agents, herbicides, insecticides, etc. The effect of the temperature factor on the cell was discussed in his report in greater detail. In the opinion of Aleksandrov (B.P. Ushakov adhered to a very similar viewpoint), in the multicellular animals and apparently in the higher plants the level of resistance of the cell proteins to high temperature is elaborated phylogenetically and is a relatively constant characteristic of the species. The change in heat-resistance of cells in the higher plants and in certain cases also in the multicellular animals can be obtained only through a considerable increase in temperature (heat toughening). The adaptation of these organisms to a change in positive temperatures during their individual life times in the absence of any excess overheating is accomplished, as a rule, by adaptive mechanisms effected at the

level of the higher stages of organization -- organ, organism and community. In the protozoans and probably in the lower plants the heat-resistance of cells is changed in accordance with the change in the environmental temperature throughout the temperature range, including the tolerance area (thermal adaptation).

B.P. Ushakov (Institute of Cytology of the Academy of Sciences USSR, Leningrad) presented the results of work on the study of heat-resistance of enzymes as well as the heat-resistance of cells in various tissues of different species of multicellular animals during natural and experimental acclimatization. It was shown that in animals raised in increased and reduced temperatures the heat-resistance of cells and enzymes is unchanged despite the change in the heat-resistance of the body. The activity of the enzymes is changed in a compensatory manner: it increases at low temperature and decreases at high temperature. Ushakov believes that we should speak of the conservative protein adaptation which is realized during the course of phylogeny and of the labile protein adaptation which is realized every day and permits the animal to exist under conditions of a changing environment.

The reports of K.M. Sukhanova (Institute of Cytology of the Academy of Sciences USSR, Leningrad), who showed the relationship of heat-resistance of parasitic protozoans to the species of host, and of P.P. Rumyantsev (Institute of Cytology of the Academy of Sciences USSR, Leningrad), who observed a constancy in the heat-resistance of the myocardium during tissue culture for three months at an altered temperature, were devoted to the problem of cell

adaptation to the temperature factor.

Yu.M. Olenov (Institute of Cytology of the Academy of Sciences USSR, Leningrad) devoted his speech to the presentation of certain general principles concerning the regulatory capacities of cells connected with the characteristics of synthesis of cell proteins and nucleic acids. The regulatory possibilities of the cell, which underlie individual adaptability in Olenov's opinion, are one of the most important objects of selection. The speaker listed a number of principles through which adaptation of the cell is accomplished to changing environmental conditions. At the same time, the speaker emphasized that the regulatory possibilities of the cell, no matter how great they might be, are of a limited nature. The only means of overcoming this limitation is natural selection of hereditary changes increasing the adaptations of the cells to changing conditions. Olenov illustrated various principles in his report with his own experimental data concerning the sensitivity of Amoeba proteus and Acetabularia mediterranea to the effect of high concentrations of amino acids.

Those speaking in the discussions presented interesting data concerning the relationship of the activity and the heat-resistance of certain enzymes of muscles and liver of fish to the temperature conditions under which the animals live (A.A. Kusakina), concerning cell adaptation in the process of tissue cultivation (S.Ya. Zalkind), concerning the absence of any effect of experimental acclimatization of frogs and actinia on the heat-resistance of ciliated epithelium (A.V. Zhirmunskiy). Yu.I. Polyanskiy, who spoke in the discussions, compared the capacity of unicellular (infusorians) animals of changing their

heat-resistance in accordance with the temperature under which they are cultivated and the conservatism of this feature in the cells of multicellular animals and plants and posed an interesting question concerning the time of occurrence of such conservatism during the course of evolution. V.Ya. Aleksandrov and B.P. Ushakov recognized the ^{importance} of solving this problem and ^{mentioned} the leading works in this direction.

At the morning session on 13 October problems were discussed of cellular permeability, bioelectrical phenomena and irritability.

The first report "The Principal Mechanisms of Cell Permeability in Connection With the Problem of Bioelectrical Phenomena and Cell Excitation" was given by A.S. Troshin. He criticized the membrane theory in its current modification, which assumes the existence of an active transportation. The principal arguments of the speaker amounted to the fact that the presence of a very large number of specifically acting pump mechanisms would have to be assumed for explaining the existing data concerning the distribution of substances between the cell and the medium, because at the present time a multitude of mineral and organic substances is known which are not uniformly distributed between the cell and the medium (under conditions of a dynamic equilibrium) and which are not subordinate to Donnan equilibrium relationships. Troshin believes that the adsorption theory better explains the entire combination of facts. According to this theory, the protoplasm represents a coacervate system in which the water is organized in a definite manner; therefore, the solubility of substances in it is less than in the water of the environment, while the cellular permeability is determined by three factors; the solubility, adsorption and chemical binding.

Any other explanation of the mechanism of distribution of mineral substances from the standpoint of sorption and membrane theories would involve a different interpretation of the bioelectrical phenomena and the excitation process. According to the membrane theory, the phenomenon of excitation consists of a change in the permeability of the membrane, whereas, in Troshin's opinion, in excitation the activity ^{of} the protoplasm itself is altered. The theoretical statements propounded in the report were reinforced by Troshin's new data concerning the kinetics of entrance of potassium into muscles and its exit into the medium which has a reduced content of this cation. The material presented attest to three forms of existence of potassium in the muscle: dissolved -- rapidly exchangeable; weakly bound -- exchangeable with lesser rapidity; and firmly bound -- the potassium of the latter fraction is exchanged very slowly for the potassium of the medium.

The second report by P.G. Kostyuk, Z.A. Sorokina and A.I. Shapovalov (Institute of Physiology of the Academy of Sciences UkrSSR, Kiev) was devoted to certain results of investigations of the physiological processes in muscle and nerve fibers carried out by a group of Kiev physiologists. In the study of the role of the ionic asymmetry in muscle fibers in a process of generating a resting potential it was shown that in the area of the physiological concentrations of potassium the relationship of the resting potential to the difference in the potassium concentrations in the fiber and in the medium is not subordinate to the Nernst and Goldman equations. In another series of experiments it was shown that the resting potential is reduced when the fiber is altered by inhibitors of oxidative phosphorylation. Kostyuk explains these data by the direct participation of metabolic processes occurring with the participation of enzymes and/macroergic phosphorus compounds associated with the conversions of in the generation of

the resting potential. The second trend of investigation reported by Kostyuk was the elucidation of the conditions under which a muscle fiber changes over into a condition of ~~xy~~ rhythmic activity. The speaker believes that the principal rhythm is constituted by oscillations of the resting potential ("prepotentials"), which are different from the ordinary action potentials and which change into the latter when a certain critical magnitude is attained. The group of works conducted under the direction of Kostyuk and devoted to establishing the relationship between the level of polarization of cells of motor and intercalated neurons and their functional state, is interesting. For the purpose of measuring the polarization level and the experimental change in it double-channel microelectrodes were used. Kostyuk's report therefore, made it possible to gain an idea of the cytological aspects of neuro-muscular physiology.

The next speaker, G.A. Kurella (Moscow University) discussed the problem of the physico-chemical nature of the resting potential. On the basis of an analysis of her own experimental material and the data in the literature Kurella stated that the resting potential, when tapped by an intracellular electrode, cannot be the result of cell alteration, because if the diameter of the electrode tip is not too great the difference in the potentials obtained does not depend on the dimensions of the area injured or on the filling of the electrode. Kurella, like Troshin, believes the explanation of bioelectrical phenomena given by the membrane theory is unsatisfactory. In searching for a non-membranous electrochemical model of a cell the speaker investigated the synthetic ion-exchange resins and showed experimentally that when the microelectrode is introduced into the resin granule interphasic potential

differences may be obtained which, like the resting potential, depend on the salt composition of the solution in which the resin and cell have been placed. Since, the potential difference tapped in such a system depends on the saline composition of the solution filling the microelectrode, and thereby the model is different from the cell, G.A. Kurella investigated another, more complex model consisting of three phases which behave similar to a cell in this respect. However, the physical explanation of the behavior of the second model proposed by the speaker apparently needs corrections.

Quite a lively discussion arose with respect to the problem of the nature of bioelectrical phenomena. N.G. Kostyuk expressed the opinion that there would be an approximation of the membrane and sorption theories in the near future. Pointing out that the proponents of the membrane theory have already given up the idea of the ^{membrane's} being a sieve, he predicted a "period of sacrifices" also for the proponents of the sorption theory. Kostyuk believes, particularly, that the alteration hypothesis for the origin of the resting potential has been finally repudiated. G.A. Kurella noted that a refusal to accept this hypothesis does not necessarily mean a refusal to accept the main position of the sorption theory with reference to the decisive role of protoplasm in the distribution of substances between the cell and the medium and in the generation of the resting potential. The localization of a potential jump at the border of the cell cannot, in his opinion, serve as proof of the exclusive role of the membrane in the generation of the resting potential. V.Ya. Aleksandrov noted the solid foundation of many critical comments made with respect to the sorption

theory. In his opinion, the direct participation of enzymes in the transportation of certain substances into the cell cannot be denied. However, the sorption theory of permeability and the denaturation theory of excitation maintain their importance as conceptions making it possible, despite specific defects inherent in them, to include quite a large group of phenomena. A.S. Troshin focused attention on the problem of the condition of the electrolytes in the cell and, particularly, potassium. In his opinion, the existence of potassium in the cell in three different forms and the absence of any correlation between the magnitude of the resting potential and the distribution of potassium (in the area of physiological concentrations of the latter in the medium), which has been shown by a number of authors, ^{is} poorly explained from the standpoint of the membrane theory. Troshin, arguing with Kostyuk, expressed his belief that the "period of sacrifices" for proponents of the membrane theory had not yet been completed. It should be noted that discussions on the problem of the nature of the bioelectrical phenomena were of a business-like and friendly nature. Among those present at the meeting the impression was created that the cause of the disagreements lay chiefly in the inadequate state of study of certain phenomena, which, because of this, can be interpreted differently at the present time.

In the report of A.D. Braun, V.L. Nemchinskaya, N.M. Nesvetayeva and V.I. Sokolova (Institute of Cytology of the Academy of Sciences USSR, Leningrad) reported new material concerning the rate of exit of certain substances from isolated muscles, including proteins, enzymes, amino acids, creatine, phosphoric acid and potassium. It was shown that in the presence of various alternative

influences the rate of exit of these substances increases.

Braun explains the data presented from the viewpoint of the denaturation theory of excitation and injury.

L.N. Seravin (Leningrad University) reported his investigations of the rhythmical activity of the infusorian *Spirostomum*. The speaker showed that the rhythmical activity of these infusorians can be produced by various agents: the effect of alcohol, salts, urea, atropine, ATP, certain dyes, sucrose and, finally, by mechanical and electrical stimulation. Seravin does not associate the occurrence of the rhythm with a disturbance in the relationships between Ca and K (or other cations), since the rhythm may be produced also in solutions free of univalent cations, but rather with an increase in excitability, which, in his opinion, always precedes rhythmical activity.

The last report heard on this day was that of A.P. Dyban and V.A. Zhuravlev (L'vov Medical Institute) concerning a method of lyophilization of tissue which they worked out which does not interfere with the intravital distribution of neutral red in the cell, making it possible to study the paranecrotic reaction of the cell in permanent preparations. It was noted in the discussion that this method opens up new perspectives in the study of paranecrosis, particularly of its early stages.

The evening session on 13 October was opened by the report of N.M. Emanuel (Institute of Chemical Physics of the Academy of Sciences USSR, Moscow) on the topic: "The Free-Radical Mechanism of Conversion of Normal Cells Into Tumor Cells". The speaker presented an original hypothesis concerning the

malignifying effect free radicals on the cell arising from the influence of various carcinogenic factors. Once they occur in the cell, the free radicals, by virtue of the principle of the indestructibility of the free valence gradually are included in progressively newer reactions, including and altering the entire cell metabolism, in the final analysis, and converting such a cell into a tumor cell from a normal one. The idea of the free-radical mechanism of the tumor process has made it possible for the author to advance the idea of utilizing non-toxic inhibitors of radical processes -- primarily inhibitors of chain oxidative processes (antioxidants) as one of the rational principles of cancer chemotherapy. In answering questions the speaker emphasized the fact that the presence of a prolonged latent period in the formation of malignant tumors does not contradict the hypothesis which he has proposed, since the free-radical type of metabolism which occurs in the cell can be maintained for an infinitely long time in principle. As far as the characteristics of the inhibitors of the radical reactions proposed are concerned, they are completely non-toxic, but, according to existing preliminary data, they possess a certain mutagenic effect.

Emanuel's report evoked a lively discussion. A.I. Serebrov emphasized that the problem of malignant growth has ceased to be merely a medical problem. Therefore, the cooperative work of biologists, physicists and chemists in the field of oncology can only be welcomed. S.N. Aleksandrov noted the great importance of new ideas, new points of view and new working hypotheses for oncology. However, as I.B. Zharskiy emphasized, the speaker's viewpoint

is very disputable. It is hard to agree that everything is chemically altered^a in/cancer cell, as is postulated by the hypothesis proposed. The great duration of the latent period in carcinogenesis has not been satisfactorily explained from the viewpoint of the free-radical mechanisms.

The report by T.G. Khaleyeva (Institute of Experimental and Clinical Oncology of the Academy of Medical Sciences USSR, Moscow) was devoted to the investigation of the effect of new antineoplastic preparations on tumor cultures and on normal human tissues. It was shown that preparations of the new type -- peptides of sarcolysin or n-di-(2-chlorethyl) aminophenylacetic acid possess a considerable selectivity of effect: tumor cells die under the influence of the peptides at concentrations two to four times less than concentrations which are lethal for normal cells. The selectivity of the effect of these peptides is greater than for embiquine, Thio-Tef and sarcolysin.

I.B. Zbarskiy (Oncological Institute imeni P.A. Gertzen, Moscow) presented data concerning the biosynthesis of protein in the nuclei of normal and tumor cells which he obtained in conjunction with K.A. Perevoshchikova. It was shown that the nuclei of tumor cells take up amino acids in vivo less actively and are different from normal cells in the distribution of radioactivity among the nuclear proteins. The authors showed the uptake of amino acids also in proteins of isolated cellular nuclei of the liver, spleen and rat sarcoma. This process was suppressed by metabolic inhibitors and by sulfhydryl compounds. Under certain conditions it was possible to show that the uptake of tagged amino acids was associated with an increase in the protein nitrogen,

that is, it reflected the biosynthesis of protein.

In the report by A.V. Gutkina and A.V. Talalayeva (Institute of Biological Physics of the Academy of Sciences USSR, Moscow) the great possibilities of fluorescent^{ce} microscopy were demonstrated both in the study and in the practical cytological diagnosis of tumors and their metastases.

At the morning session on 14 October reports were presented on radiation cytology. In the report by M.N. Meysel', G.A. Medvedeva and M.N. Pogliazova (Institute of Microbiology of the Academy of Sciences USSR, Moscow) certain rules and regulations were discussed with respect to the death of cells directly during irradiation, the condition of cells after various doses of radiation, as well as characteristics of the reaction of the mitochondria to irradiation. In the opinion of the speaker (M.N. Meysel'), radiation injury of the cytoplasm plays an essential part in the general radiobiological effect and specifically exerts an unfavorable effect on the nucleus (in his concluding statement, however, M.N. Meysel' made it clear that the injury to the cytoplasm is of essential importance only in acute radiation injury), in spite of the views of B.L. Astaurov. In answering a question concerning the relative role of the direct and indirect effects of radiation on the cell, M.N. Meysel' emphasized that great importance should be ascribed to the direct effect, apparently, in connection with the latest ideas of the particular state of water in the cell, which considerably restricts the sphere of action of the free-radical mechanisms.

V.P. Paribok (Institute of Cytology of the Academy of Sciences USSR, Leningrad) suggested a single explanation for the protective effect of anesthetics both at the level of the intact animal organism and at the level of the cell in his report, namely: the adsorptive displacement of oxygen molecules from cell surfaces and a reduction in the concentration of the primary products of aerobic water radiolysis.

N.I. Shapiro (Institute of Biological Physics of the Academy of Sciences USSR, Moscow) presented experimental data on the study of the effect of protective substances at the cell level. Utilizing the frequency of chromosomal aberrations in the irradiated mouse Ehrlich carcinoma as a criterion, the speaker showed the protective effect of streptomycin (at certain concentrations) and of the narcotics nembutal and heroin.

M.P. Bukhman and T.M. Kondrat'yeva (Optical Institute imeni S.I. Vavilov, Leningrad; Central Scientific Research Institute of Medical Radiology, Leningrad) investigated the effect of ionizing radiation on the bone-marrow cells of animals by the method ^{of} ultra-violet microscopy (utilizing an MUF-2) on living and fixed preparations. They showed that this effect is expressed in an increase in absorption of ultra-violet rays with a wave length of 365 millimicrons and in an increase in these size of the cells. It was also shown that fixation in formalin vapors introduces a number of artefacts into the picture of injury (this latter assertion was disputed by V.Ya. Brodskiy, who spoke in the discussions).

In a report by L.I. Il'yina (Academy of Medical Sciences, Moscow) certain aspects of protein metabolism in the cell organoids in acute radiation

sickness were discussed. The data presented attest to the profound disturbance of protein metabolism, particularly of the occurrence of proteins which are altered in an antigenic respect, possessing a high degree of toxicity.

The speech made by Ya.L. Shekhtman in discussions was interesting. In his opinion, the ^{current} ideas of the physical state of water in cells and its connections ^{with} the biomolecules make impossible any direct transfer of the laws of radiation chemistry obtained through the investigation of aqueous solutions in vitro to the processes which are unfolded after irradiation of the living cell.

Therefore, any ideas of the indirect effect of radiation on the biomolecule through the formation of free radicals in an aqueous phase (see, for example, Paribok's report), in Shekhtman's opinion, should be considered as compromised. In this connection, the problem of the direct effect of radiation on the cell and primarily on its nucleus should be reexamined. Specifically, factual data presented in Meysel's report, in Shekhtman's opinion, constitute evidence precisely to the effect that the cytoplasm does not play any part in the developing radiation injury. S.N. Aleksandrov, by and large, agreed with Shekhtman's opinion.

Ye.G. Zinov'yeva, who spoke in discussions on Meysel's report noted that in the investigation of cytoplasmic and nuclear injuries of the cell after irradiation it is absolutely necessary to take into consideration the fact that the products of radiolysis of the medium (particularly water) in which the cell is being irradiated are very toxic and in themselves can serve as the cause of

serious injuries to the cytoplasmic structures.

Finally, in connection with Paribok's report, the problem of the oxygen effect in irradiation was touched on in a number of talks.

At the evening session B.L. Astaurov's report was given first (Institute of Animal Morphology imeni A.N. Severtsov of the Academy of Sciences USSR, Moscow) concerning polyploidy and parthenogenesis in the silkworm. The author demonstrated a system of obtaining parthenogenetic triploid females, which after crossing with diploid males lay about one percent fertilized eggs, which develop into ~~triploid~~ tetraploid males and females. It was made clear cytologically that in the development of the parthenogenetic triploid females a frequent polyploidization occurs which leads to the formation of both somatic and sexual hexaploid cells.

After reduction division the latter contain the triploid female pronucleus and as a result of karyogamy with the haploid male pronucleus form tetraploid zygotes, which develop into individuals of both sexes. The tetraploid females are fertile; the tetraploid males, on the other hand, are absolutely sterile. One of the probable causes of sterility of the males is considered the aneuploidy /absence of the proper number of chromosomes/ of their sperm as a result of the abnormal behavior of the chromosomes in meiosis.

The next reports were devoted to an examination of pollen granules and pollen tubes. The report by M.S. Navashin, L.M. Makushenko and Z.V. Bolkhovskikh (Botanical Institute imeni V.L. Komarov of the Academy of Sciences USSR, Leningrad) was accompanied by a demonstration of an original ... colored film beautifully

made by the method of high-speed microfilm photography, which lent special weight to the authors' conclusions. Through the example of several species of *Amarillis* it was shown that the vegetative nucleus and the generative cell change into the pollen tube during its growth at a time when half or more of the cytoplasm has left the pollen granule. The vegetative nucleus precedes the generative cell. Movement of the cytoplasm in the tube is accomplished by two countercurrents. The generative cell is always in the ascending current of the cytoplasm and is moved passively in it.

In the next report by V.A. Poddubins-Arnol'd, Ye.V. Tsinger, T.P. Petrovskaya and N.N. Polunina (Main Botanical Garden of the Academy of Sciences USSR, Moscow) devoted to the histochemical investigation of pollen and pollen tubes in certain plants, the speaker T.P. Petrovskaya reported that pollen and pollen tubes in 64 species investigated contain a large quantity of proteins, amino acids and fat. A high degree of activity of catalase, cytochrome oxidase and peroxidase is characteristic of them. In many plants ascorbic acid and heterauxin were found. Considerable dehydrogenase activity was observed chiefly in the pollen tubes; however, a considerable variation occurs in the content of the substances investigated even in the pollen used from a single anther. A particularly high content of proteins, RNA, amino acids, enzymes and other physiological reactive substances was noted in the apices of the pollen tubes, which indicates a high level of activity.

The next two reports were devoted to a study of nuclear structures.

I.I. Sokolov (Institute of Cytology of the Academy of Sciences USSR, Leningrad)

reported on the results of a comparative cytomorphological investigation of the nucleolar apparatus during the oogenesis of spiders. In material which included 17 species the species specificity of the morphological characteristics of the nucleoli was determined as well as their changes during the course of the period of considerable growth of the oocytes. These changes were considered by the speaker to be a morphological expression of the tendency toward an increase in the general activity of the surface of the nucleolar apparatus. It was established that the nucleoli possess different kinds of fine inner structures and differentiation.

In the report concerning the investigation of nuclear structures of amphibian oocytes P.V. Makarov (Leningrad Sanitary-Hygienic Institute) presented his ideas on the formation and structure of chromosomes of "the lamp brushes" in the frog oocytes. The author showed changes in the structure of these chromosomes, in the content of their acid proteins and in the DNA in frogs of different ages. Contrasting his opinion with that of the majority of the investigators the speaker made the assumption that the "lamp brush" chromosomes do not show any successive connections with the figures of the bivalent chromosomes.

At the conclusion of the meeting V.K. Stepanova (Stavropol' Medical Institute) gave a report on the topic: "Determination of the Specific Nature of Tumor Cells and Cytodiagnosis of Tumors by Means of ^{Fluorescence} Microscopy".

It was made clear that cancer cells are different from normal cells in their brighter coloration and in the change in fluorescence of the cytoplasm and nucleus in the direction of the yellow-orange portion of the spectrum,

which ~~examined~~ can serve as an adequate criterion permitting the rapid diagnosis of tumor cells.

In a discussion on these reports a talk was made by I.I. Kiknadze, who dwelled on the problem of the connection of the . nucleolus and chromosomes. R.I. Salganik and N.I. Shapiro pointed out, in connection with Makarov's data, that ^a negative Feulgen reaction still does not mean the absence of DNA in the nucleus, because it may be inhibited by ions of iron. A.A. Prokof'yeva-Bel'govskiy noted the great importance of Astaurov's data, which will particularly stimulate future investigations of the causes of polyploidization. With respect to Makarov's data she emphasized that at the present time they cannot be evaluated, because the material was presented only in diagrams and needs a more careful investigation.

After the meeting a number of movie ^{films was} shown which evoked the considerable interest of the conference participants.

The morning session of 15 October was devoted to cytochemical investigations.

G.I. Roskin, B. Kozhukhova (Moscow University) gave a report on the cytoenzymology of succinic dehydrogenase. For the purpose of demonstrating this enzyme in the cells of normal and malignant tissues the authors used ^{the} tellurite method which made it possible to ^{determine} by the topography of the free tellurium granules that the succinic dehydrogenase in various cells is associated with different cell elements. In the opinion ^{of} the authors, the tellurium reaction has a number of advantages over the other histochemical

methods of determining this enzyme.

Of the group of authors, Ya.A. Vinnikov (Institute of Evolutionary Physiology imeni I.M. Sechenov of the Academy of Sciences USSR, Leningrad) gave a report on the topic "Cytochemical Investigation of the Activity of the Succinic Dehydrogenase and of Cytochrome Oxidase in the Mitochondria of Receptor Cells, Neurons and Muscle Fibers in a Condition of Relative Rest and Under Conditions of Excitation". He showed that in the neurons of the vestibular and spinal ganglia, auditory cells and certain other nerve cells in a state of relative rest the respiratory enzymes are found in the mitochondria. With excitation there first occurs a marked increase in the activity of the enzymes, a swelling and deformation of the mitochondria, which after prolonged stimulation are replaced by a decrease in the activity of the enzymes. The authors believe that the changes in the activity of the respiratory enzymes which they determined confirm the denaturation theory of D.N. Nasonov and V.Ya. Aleksandrov.

The report by V.V. Portugalov was devoted to a similar topic, "New Data on the Physiology of Mitochondria" (Brain Institute of the Academy of Medical Sciences SR, Moscow); he utilized a cytochemical method for demonstrating the connection between the localization of the succinic dehydrogenase in the mitochondria and the functional characteristics of the cell. In a combined report/L.B. Levinson and S.M. Kolomina (Moscow University), "Morphological and Cytochemical Investigations of the Mitochondria of Nerve Cells Depending on Their Functional State". It was shown that with an increase in the intensity of the specific function of the nerve cells the morphology of their mitochondria is changed, and ~~it~~ there is also an

increase in the activity of the acid phosphatase.

The report by V.Ya. Brodskiy (Institute of Animal Morphology imeni A.N. Severtsov of the Academy of Sciences USSR, Moscow), who, by means of comparing the spectral absorption curves and characterizing their indices

$= D_{\lambda 265} / D_{\lambda 280}$ (ratio of the optic densities of RNA at two wave lengths), establish the fact that the latter varies in the nucleus and various areas of the cytoplasm, was devoted to the application of the spectrophotometric method in the investigation of ribonucleic acids of the nucleus and cytoplasm of animal cells. This variation is similar in the cells of different types and in animals even of distant species. The speaker concluded that the change in the β -index in various portions of the cell can be explained by differences in the nucleotide composition and by the different constructions of the various RNA molecules, but is not associated with either with the lack of uniformity in distribution of the chromophores or with the different degrees of polymerization of RNA in the cells.

R.I. Salganik, T.M. Morozova and I.I. Kiknadze (Institute of Cytology and Genetics of the Academy of Sciences USSR, Novosibirsk) reported on the biochemical research done on the isolated nuclei of the thymus. The authors confirmed the data of Mirsky and others to the effect that, along with a reduction in the DNA content, the intensity of uptake of tagged amino acids and nuclear proteins is reduced in such nuclei under the influence of desoxyribonuclease as well as the fact that the capacity of nuclear proteins for taking up tagged amino acids is restored after homologous(or heterologous) DNA or RNA ^{is} introduced into a medium where such

nuclei are being incubated. The authors showed later that this recovery apparently can be attributed to a resynthesis of their own DNA, which has not been completely destroyed by the effect of desoxyribonuclease, by the nuclei.

a report was given by

At this meeting O.A. Vasilëva (Bulgarian cytologist);

she presented data concerning the DNA content in the cells of the embryonic sac in plants of the lily family.

M.N. Meysel', G.I. Roskin and Ya.A. Vinnikov, who spoke in the discussions discussed the possibility of demonstrating succinic dehydrogenase in the mitochondria by the tetrazane method. Yu.M. Olenov gave high praise in his report to the work of R.I. Salganik and co-authors noting that they not only have confirmed the well-known data of Mirsky and others but have also made a deeper analysis of the phenomenon observed.

At the evening session on 15 October Yu.I. Polyanskiy and I.B. Raykov (Institute of Cytology of the Academy of Sciences USSR, Leningrad) gave a report on the role of polyploidy in the evolution of protozoans. The authors pointed out that in the protozoans two categories of polyploidy can be distinguished. In the micronuclei of a number of infusorians, ^{and} in the nuclei of Hyper- and Polymastigina a relatively low degree of polyploidy occurs ($4n-8n$). It is not associated with disturbances in the ordinary mechanism of mitosis. The macronuclei of the infusorian and the original nuclei of certain radiolarians are characterized by very high degrees of polyploidy (up to thousands of n) and by a replacement of mitosis by a special form of nuclear division ("segregation of genomes" -- Grelle), which represents a distribution of entire chromosomal sets among the daughter nuclei.

A comparative study of nuclear apparatus of lower infusorians makes it possible to state that the nuclear dualism and polyploidy of the macronucleus are within limits of the class of infusorians. Thereby, the occurrence of a high degree of polyploidy of the macronuclei may be regarded as a progressive line in the evolution of protozoans connected with the accumulation of large quantities of nucleoproteins and the intensification of the functions.

The report of A.A. Imshenetskiy (Institute of Microbiology of the Academy of Medical Sciences USSR, Moscow) which he presented in conjunction with G.A. Zavarzin and V.V. Alferov was devoted to the problem of the nucleus in true bacteria (Eubacteriales). The speaker repeatedly defended the viewpoint which he had previously expressed, according to which there is no specialized nucleus in the true bacteria, and the DNA which exists is distributed in the cell diffusely. He criticized methods of treating preparations extensively used at present for demonstrating the nuclei in true bacteria. According to the data of the authors' report, the nucleoids demonstrable by these methods in non-sporogenic bacteria ~~contain~~ swollen and displaced polar granules.

To the question of the role of the data on bacterial genetics in proving the presence or absence of nuclei in them Imshenetskiy answered that in his opinion these data, although very interesting, are still very few and not completely convincing.

As is well known, M.A. Peshkov (Institute of Revolutionary Morphology imeni A.N. Severtsov, Moscow) has for a long time now been an opponent of the views of Imshenetskiy on the question of a nucleus in bacteria. However,

this time in his report, which was devoted to the current concepts of the bacterial structure, he avoided the term "nucleus", but rather spoke of the nucleoids, nuclear structures, etc. The report was accompanied by a demonstration of a large number of diapositives and microfilms on the morphology of bacteria.

A.A. Prokof'yeva-Bel'govskaya (Institute of Biological Physics of the Academy of Sciences USSR, All-Union Scientific Research Institute of Antibiotics, Moscow) reported on the data which she had obtained in conjunction with O.N. Kapitonova, G.R. Mikhaylova and Z.B. Shamina concerning the cytology of actinomycetes. Through comprehensive investigation on the cytology, cytochemistry, biochemistry and physiology of the actinomycete cell the characteristics of its chemical composition, microscopic structure and the properties of the cytoplasm and nuclear elements were clarified. A close relationship was established between the microscopic morphology and nature of functioning of the cell (creation of antibiotic). It was shown that during the period directly preceding a visible division of nuclear elements the cell is most sensitive to irradiation. Changes in the properties of the nuclear elements (mechanism of their division, content of DNA in them) underlie the numerous changes in the cell occurring as a result of irradiation.

The report by N.A. Krasil'nikov and L.V. Kalakutskiy (Institute of Microbiology of the Academy of Medical Sciences USSR, Moscow) was devoted to a cytomorphological study of the anaerobic proactinomycetes. The data obtained did not confirm the opinions of a number of foreign authors concerning the

presence of a complex development cycle or of spore formation in these organism, etc.

The discussions unfolded chiefly on reports of Imshenetskiy and Peshkov.

M.N. Meysel' noted that the previous alternative -- the presence or absence of a nucleus in bacteria -- might perhaps have disappeared, since Peshkov now speaks only of nucleoids or nuclear structures but not of a nucleus as he had previously. In Meysel's opinion, it is necessary to review the criteria of the nucleus in cytology. At the same time, according to all the basic structural, chemical and functional features the nuclear structures in bacteria should be considered a nucleus. In the opinion of A.A. Prokof'yeva-Bel'govskaya the nucleus of the higher organisms and the nucleus of bacteria cannot be completely identified, because the concept of chromosomes, nuclear membrane, etc. are ^{indisruptibly} associated with the concept of a nucleus.

In striving for an accurate scientific terminology we cannot call a structure at various stages of evolution by the same terms. S.I. Alikhanyan in his talk ^{the} emphasized that most essential in the concept of a nucleus is/ biochemical and functional characterization. In this connection, the data of bacterial genetics undoubtedly speak for the presence of a nucleus in them which is similar to the nucleus of higher organisms.

At the end of the meeting the director of the Institute of Cytology of the Academy of Sciences USSR, A.S. Troshin, presented ^{some of the} results of the conference. Touching briefly on the history of organization of the present conference, he presented some of the data and figures attesting to the great

interest in this measure on the part of biologists, physicians as well as physicists and chemists. Later, Troshin analyzed the thematics of works presented at the conference which have been published in the form of a collection. The thematics and number of reports to a certain degree reflect distribution of forces Soviet cytology. In this connection, the absence in the conference program of any special meeting on the microscopic and submicroscopic organization of the protoplasm attracts attention primarily. The number of works on the cytology of malignant growth, on the problem of permeability and the nature of biopotentials is clearly inadequate. Relatively few works have been accomplished on plant cells. In the conference program there was no special meeting devoted to methods of cell investigation; there were few works carried out with the use of tagged atoms, microelectrode and micrurgical technique, electronic, ultra-violet and fluorescence microscopy. All this apparently speaks for a certain lag in these areas of cytology. In conclusion Troshin acquainted those present with the structure and tasks of the Scientific Council on the Problem "Key Questions of Cytology" and posed the question for discussion of the expediency of organization of a society of cytologists.

The next three meetings were expanded meetings of the Scientific Council of the Academy of Sciences USSR on the problem "Key Questions of Cytology". The first of them was devoted to informing the participants of the conference concerning research on cytology being conducted at various places. Yu.I. Polyanskiy (Institute of Cytology of the Academy of Sciences USSR, Leningrad)

gave a general introductory report on the tasks of the Scientific Council and the principal trends of the work being performed in the Institute of Cytology and in a number of other scientific and teaching institutions in the country. The speaker pointed out that the forthcoming exchange of information will contribute to a clarification of the condition of cytological works, which undoubtedly will facilitate the task of coordinating cytological investigations.

In the speeches which then followed given by a number of participants of the conference a report was made concerning the research being conducted in the institutions which they listed on cytology as well as on certain conditions and defects in the research work.

The second session of the Scientific Council on the problem was devoted to a discussion of the results and tasks on the investigation on some divisions of cytology.

The last meeting of the Scientific Council was begun with a discussion of the first issues of the journal Tsitologiya. The main editor of the journal, A.S. Troshin gave information concerning the work of the editorial college and the contents of the first few issues of the journal. In discussing the report 14 persons participated who noted a series of defects and expressed many valuable desires with respect to editing the journal.

Then, the resolution of the First Coordination Conference was discussed and adopted unanimously. In the resolution it was noted that in recent years favorable conditions have been created in the Soviet Union for increasing the work in the area of cytology, and in working out a number of

divisions in cytology considerable progress has been made. This applies to problems of cytoecology, radiation cytology, cytophysiology (problems of irritability and permeability), the cytology of plant fertilization, intravital investigations of microorganisms by moviefilm methods with special methods of microscopy and some others. Substantial results have also been achieved in the study of the interrelationships between the nucleus and cytoplasm both by methods of cytoembryology and by methods of cytochemistry. Works on cytochemistry and on the application of modern methods of tissue cultivation and cell cultivation have been revived noticeably in recent years. The combined work of cytologists and optical physicists and workers in the optical industry has led to the development of a number of original devices and new methods of microscopy.

At the same time, it was noted in the resolution that a number of the sections of cytology is being developed inadequately and lags behind the level achieved by this science in certain foreign countries.

The coordination conference, after acquainting the participants with the state and development of various scientific trends presented at the conference recognized the following as necessary:

1. That research in microscopic and submicroscopic organization of the cell with the application of special current methods of investigation and primarily of electron microscopy be increased markedly.
2. That work be intensified on the study of cell reproduction and elementary cell structures at the microscopic and submicroscopic level.

That special attention be ^{directed} to the study of morphology, physiology and biochemistry of mitosis with the utilization of antimetabolites and tagged compounds.

4. In connection with the extremely ^{inadequate} development of research in special fields of physiology and cell morphology works should be expanded on problems of fertilization, growth, differentiation, contractile and secretory functions of the cell.

4. Work should be extended on a broader scale concerning the most important problems of cell physiology, irritability, permeability and cellular adaptations.

5. In the near future work should be increased in the field of morphology and physiology of the plant cell, as well as investigations in the field of morphology, physiology and biochemistry of the processes of malignant degeneration and the study of the interrelationship of viruses and host-cells.

6. In connection with the expansion of the application of atomic energy and the increase in the possibility of radiation injury research should be developed ^{on a broader} ^{scale} in the area of radiation cytology. It is also necessary ^{the} to increase the study of bacterial cell and of unicellular organisms.

7. The conference notes the essential significance of the experimental cytological research on the problem of development and heredity and also directs special attention to the necessity for creating new methods of cytological research and improving those used at the present time.

For purposes of the further development of cytological research in the USSR the First Coordination Conference considers the following measures to be of first importance:

1. Coordination scientific conferences for the purpose of discussing key questions of cytology, planning and coordination of scientific works to be accomplished in various scientific groups should be held periodically (once every two or three years); the Scientific Council should be given the organization of symposia on the most important cytological problems and on those which are being worked on most intensely and, particularly, a symposium should be held in 1960 devoted to the role of denaturation phenomena in physiological processes.

2. In connection with the fact that at the present time the requirements made on young specialists in the field of cytology have increased, the most serious attention should be given to the training of cytological personnel of broad profile /specialized in a large field/. Teaching of cytology should be expanded in the medical and pedagogical institutes. Taking into consideration the fact that none of the existing textbooks on cytology can be considered satisfactory it is essential to set about preparing a Soviet textbook on cytology as a basis for the training of young cytological specialists.

In the resolution the great beneficial significance of the creation of the journal Tsitologiya was noted for the development of cytology and the necessity of increasing its volume, improving its formulation and for publishing a resumé of the articles in a foreign language. In the resolution the

necessity was mentioned for ^{strengthening} the material foundation for cytological research. A problem of first importance in this direction should be the production of better microscopes and of the various adaptations for it, a radical improvement of existing types of microtomes, the construction and production of apparatus for lyophilization, various centrifuges, separators for separating the cell components. The creation of special electrical measuring and electronic apparatus for cytophysiological research [sentence incomplete in original text]. It is also essential to provide laboratories with a broad assortment of standard chemically pure reagents, stains and other compounds.

At the conclusion of the First Coordination Conference the general belief was expressed that the accomplishment of all the tasks noted in the resolution will make it possible for Soviet cytology to ^{make} great scientific achievements.

During the conference at the Institute of Cytology a demonstration of cytological preparations was held and exhibits of new models of microscopes and the latest Soviet and foreign literature on cytology ^{were} operating continuously, which was very successful with the participants of the conference.

Some Biological Institutions of Modern France

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By way of a cultural exchange between the Academy of Sciences USSR and the National Scientific Research Center of France (Centr. National de la Recherche Scientifique, abbreviated CNRS). The author of this article was sent on a two-month's trip on scientific detached service ^{to} France (from the end of November 1958 through the end of January 1959). The detached assignment pursued a double aim: 1) acquaintance with certain biological institutions of France, 2) scientific work at the biological station at Roscoff (Brittany) with respect to a topic being worked out at the Institute of Cytology and dealing with a comparative study of the nuclear apparatus of protozoans.

In the present article I should like to give a brief characterization of the organization of scientific work in the field of biology in France and to discuss briefly the activity of certain scientific biological institutions. It goes without saying that for the relatively short time of my detached service assignment I was able to become acquainted with only several laboratories. For three weeks I was in Paris and for a month I was at the biological station at Roscoff. In addition, I had the opportunity of visiting the biological station at Banyuls (Mediterranean Sea, French Pyrenees) and the newly organized University at Rheims.

In all the institutions which I visited I was given an

exceptionally friendly reception by my French colleagues. I should particularly like to express my appreciation to those French colleagues who gave me particularly great aid and assistance. These were Professors Teissier (The Sorbonne), Prenant (The Sorbonne), Lamotte (The Sorbonne), Fauré-Fremier (CNRS), Ephrussi (The Sorbonne) and CNRS), senior scientific worker of the Pasteur Institute, Gelin, Professor Goncharova (University of Rheims), Professor Petit (Director of the Biological Station at Banuyls) Professor Dollfus (Museum of Natural History).

During my stay in France I became acquainted with the work of the following scientific biological institutions: The Biological Station at Roscoff (Director, Professor Teissier), The Biological Station at Banuyls (Director, Professor Petit), the chair of invertebrate zoology of the University of Paris (Director, Professor Teissier), the chair of comparative anatomy and histology of the University of Paris (Director, Professor Prenant), chair of evolution of organisms of the University of Paris (Director, Academician Grassé), the genetics laboratories of the Scientific Center (CNRS) at Gif' (physiological genetics -- Professor Ephrussi; evolutionary genetics and biometry -- Professor Teissier; formal genetics -- Professor L'Héritier), the laboratory of parasitology of the medical faculty of the University of Paris (Director, Professor Shabaud), the laboratory of parasitology of the Museum of Natural History (Director, Professor Dollfus), the laboratory of animal biology of the École Normale Supérieure (Director, Professor Lamotte), the laboratory of cytology of the Scientific Center (Director, Professor Fauré-Fremier), and the laboratory of viruses of the Pasteur Institute (Director, Professor Lwow).

The biological sciences in France are being developed chiefly in the universities, at the numerous biological stations (the majority of them are connected with the universities). The national scientific center (CNRS) plays a special part in the development of biology (as also in the other sciences), and its importance is increasing in France from year to year. The CNRS is a state institution the task of which is to contribute to the development of science. As far as the biological sciences are concerned, the CNRS, on the one hand, has its own scientific research institutions, and on the other hand, gives aid to other institutions (chiefly to universities in their biological stations) in the form of assignments for the acquisition of scientific equipment. In addition, the CNRS gives scientific placements (for three to five years according to contracts) to scientific workers. The CNRS invites also foreign students for working in the scientific institutions of France and for giving reports, organizes international scientific colloquia and finances many scientific publications. As an example it may be pointed out that during the period from October 1956 through October 1957 10 such colloquia were organized, including three on matters of biology and medicine.

The total number of scientific workers in the various ranks working in the CNRS system in 1958 was equal to approximately 1500 (in all sciences, including the humanitarian sciences); the number of scientific technical workers was approximately the same.

practically
In contrast to the Academy of Sciences USSR there is/no permanent staff
f workers in the CNRS system (functions of which to a certain degree are similar

to the Soviet Academy). Usually a contract is made for various periods of time (three to five years) with individual scientific workers after the introduction of them by scientists; for the renewal of this contract a new introduction is required. The workers who carry out their work according to the CNRS contracts are not state employees ("fonctionnaires"), and this reflects unfavorably on their pension provisions -- on reaching pension age they do not receive pensions, which are awarded to scientific workers in the universities in France. Therefore, mature and elderly scientists usually attempt to change from the CNRS system to staff work in the university institutions.

At the present time, the following biological institutions are included in the CNRS system:

1. Laboratory of Biology of the Plant Station for the Study of Cold (Professor Ulrich, Belleville).
2. Laboratory of Biochemistry of Digestion (Professor Jacquot, Belleville).
3. Laboratory of Evolutionary Genetics and Biometry (Professor Teissier, Gif-sur-Yvette).
4. Laboratory of Formal Genetics (Professor L. Héritier, Gif-sur-Yvette).
5. Laboratory of Physiological Genetics (Professor Ephrussi, Gif-sur-Yvette).
6. Laboratory of Photosynthesis (Professor Moiseaux, Gif-sur-Yvette).
7. Laboratory of Phytotron [?] (Professor Jouart, Gif-sur-Yvette).

8. Laboratory of Hydrobiology (fresh-water) (Professor Lefèvre, Gif-sur-Yvette).
9. Selection Center of Laboratory Animals (Rabourdis, Gif-sur-Yvette).
10. Investigation Center of the Sahara (comprehensive laboratories, including biological group).
11. Investigation Center of Nerve Physiology and Electrophysiology (Professor Fessard, Paris).
12. Laboratory of Embryology and Experimental Teratology (Professor Wolf, Collège de France, Paris).
13. Laboratory of Experimental Morphology (Animal) (Professor Avelle, Bordeaux).
14. Investigation Center of Man (Chiefly of Labor Physiology; Professor Sulat, Paris).
15. Laboratory of Biometry (Professor Logier, Paris).
16. Center of Nutritional and Food Products Research (Professor Terruant, Paris).
17. Laboratory of Nutritional Physiology (Randouin, Paris).
18. Laboratory of Electron Microscopy in its Application to Biology (Academician Grassé, Paris).
19. Laboratory of Subterranean Research (Professor Wandel, Toulouse).
20. Center of Oceanographic Research and Sea Biology (Laboratory Ives Delage, Professor Teissier, Roscoff).

21. Institute for the Study of Cancer (Professor Oberlin, Villejuif).

22. Phytogeographic Map Service (Professor Emberger, Montpellier).

The list of scientific institutions of the CNRS presented above shows that principally work is being conducted in the field of the various divisions of experimental biology. The problems of faunistics, classification and biogeography are practically not within the sphere of attention of the CNRS.

Aside from universities and institutions of the CNRS, scientific works in the field of biology are being carried out also in various other institutions. The faunistic and floristic research in large part is concentrated in the National Museum of Natural History. A number of interesting works in various fields of biology has been concentrated in the great scientific institution -- the Pasteur Institute (microbiology, virology, immunology, medical parasitology and entomology), as well as in various institutions which are of scientific practical nature (in the system of the Ministry of Health, in the Academy of Medical Knowledge, in the Center of Agronomic Research, etc.

It should be noted that research work of universities (particularly of the University of Paris) and of the CNRS are exceptionally closely intertwined and at times practically cannot be separated. The same leading French biologists are the directors of the university chairs and the laboratories of the CNRS. The scientific personnel in large part are also the same. Very frequently, in laboratories one part of the equipment belongs to the CNRS; another to the university, etc. For example, the biological station at Roscoff is an institution included in the system of the faculty of sciences of the University

of Paris. At the same time, at this station there is based a center of oceanographic research of the CNRS. The personnel of the station is supplied partly from the CNRS and partly from the university. This example is very typical, and it is repeated in various modifications in all the biological scientific institutions of France. Therefore, in the presentation which follows in characterizing the scientific work of some biological institutions of France I shall, in a number of cases, have to begin with the trend of the research, associated usually with the professor directing the laboratory, rather than with the administrative-organizational category of the institution.

Let us turn to a characterization of the scientific activity of certain other research institutions of the CNRS located in the vicinity of Paris (at Gif-sur-Yvette). These laboratories are closely associated with the chair of invertebrate zoology of the Sorbonne, so that we shall speak about them together.

The director of the chair, Professor Teissier, one of the outstanding French zoologists, is at the same time director of the laboratory of evolutionary genetics and biometry of the CNRS Gif-sur-Yvette. Teissier's research and that of his colleagues in population genetics, which was carried out using Drosophila melanogaster, is of great interest. Teissier has worked out an original experimental method of creating populations in large groups, making it possible to accurately control the conditions of their development and their genetic structure. In these experimentally created populations various mutant strains have been introduced, and in prolonged experiments a study is being made of their interrelationships with the "normal" forms of Drosophila. Here, by regulating the

nutrition, conditions are created for different degrees of intensity of competition and a study is made of the dynamics of natural selection and the change of the genetic structure of the populations with time. By this method many results have been obtained which are of importance in principle for understanding the mechanism of action of natural selection. A study was made of the rapidity of the change in genetic structure of populations, the conditions of preservation of heterozygotic forms, the evolution of complex polygenic systems, the course of natural selection with change in the environmental conditions, etc. These investigations, which enjoy great renown, are being continued at the present time both in the laboratory at Gif and at the chair. In recent years this method has been used for the study of the geographic races of *Drosophila* and for an analysis of selection rating of the internal anatomical characteristics, for example, the number of oviducts. The research of one of Teissier's co-workers, Claudine Petit, devoted to the problem and mechanism of very sexual selection in *Drosophila* are interesting. By means of very fine experiments she succeeded in showing not only the fact itself of sexual selection but also in elucidating its quantitative aspect. Research in the field of population genetics and of the genetic structure of the species is being carried out in Teissier's laboratories not only with the *Drosophila* material. A study is being made along this line of certain crustaceans (*Sphaerosoma serratum*) (Teissier, Bocquet and Levi), *Jaera marina* -- Bocquet) and molluscs -- Lamotte. Recently, a number of works have also been carried out on the study of heterosis.

Along with the genetic-population research in the laboratory of evolutionary genetics at Gif a number of cytogenetic investigations is being accomplished, of which the works of Bergerard on cytology of parthenogenetically multiplying insects from the Phasmidae group are particularly interesting. In them the entire picture of the changes in the chromosomal apparatus is presented in detail both in the development of gametes and in the course of ontogeny.

Along with the experimental-genetic study of populations Teissier has been occupied with the study of the rules and regulations of animal growth, chiefly with arthropods. These works constitute a detailed quantitative analysis with the application of complex biometric methods. Teissier has succeeded in giving a mathematical expression to the rules and regulations of growth, which is of great biological interest.

An important place in the scientific ^{work of the chair} / and laboratory headed by Teissier is occupied by research on the postembryonic development and endocrinology of arthropods. These investigations, which enjoy great renown, are at the present time being continued very actively. Among them note should be made primarily of the work of Posompés on the metamorphosis of insects. In *Dixippus* midges he experimentally showed the relationship of the advent of molting to the endocrine glands adjacent to the brain -- the corpora allata. He worked out a very fine and accurate method for surgical operations, making it possible to remove the corpora allata and thereby to elucidate their role in the processes of molting. By using the method of extirpation and transplantation Posompés succeeded in clarifying the complex relationships in the metamorphosis

in certain diptera (in particular detail in Calliphora erythrocephala) to the system of endocrine glands associated with the brain (peritracheal glands, the "Weismann ring", the corpora allata) and of showing the phenomenon of neurosecretion. All these investigations were the most important phase in the study of the postembryonic development and metamorphosis of insect. The technique of very fine brain operations on insects which Posompés mastered with the art of a virtuoso was demonstrated to me.

In this series of investigations of the endocrinology of arthropods are also the works of Charmiaux-Cotton, on crustaceans. She succeeded in demonstrating the existence of a particular gland of internal secretion -- the androgenic gland (glande androgène) associated with the sperm ducts in the male ~~side-swimmer~~.

The secretion of this gland determines the development of the entire complex of male secondary sex characteristics. By the method of transplantation Cotton was able to achieve a complete change of sex. These investigations posed the problem anew concerning the mechanism of development of sex characteristics in crustaceans ~~which~~ are of great importance in principle for the physiology of development of sex characteristics. With respect to these works it is also essential to emphasize that the technique of microscopic operation is on a very high level. The animals themselves ~~(side-swimmers)~~ have diameters of about one centimeter, and it is a very difficult matter to perform ~~the~~ operation of transplanting glands of microscopic size in them.

The experimental research of Dupont-Raabe devoted to the experimental

analysis of the change in color in insects are contiguous with this series of works. She was able to show that these changes, which frequently are of an adaptive nature, are carried out under the control of the nervous system and the glandular apparatus associated with it.

In addition to what has been analyzed above, a number of works are being accomplished in the laboratories of Teissier by his co-workers and students; the majority of these works are associated with one of the trends listed above.

Investigations being carried out in the laboratories directed by Professor Ephrussi at the Sorbonne and Gif (CNRS -- laboratory of physiological genetics) are of great interest. The majority of them are devoted to the genetics of microorganisms. Here, extensive use is being made of cytochemical and biochemical methods. The investigations are being carried out on a very high technical level. Methods of cultivating various microorganisms have been worked out (including fungi--ascomycetes and basidiomycetes), making it possible to control accurately the conditions of the medium. One of the principal problems being worked on in the laboratories of Ephrussi is the problem of the interrelationship of the nucleus and cytoplasm in the phenomena of heredity. In this direction the works of a co-worker of Ephrussi, Slonimski, devoted to the study of the respiratory processes in yeasts as determined by cytoplasmic factors, are of great interest. Slonimski's and Tysarowski's investigations on the transformation of the respiratory enzymes

of yeasts is particularly interesting. The authors have managed, by means of adaptation to environmental conditions, to convert an anaerobic enzyme into an aerobic enzyme and to observe its subsequent hereditary transmission.

Of great interest in principle are also the experimental investigations being carried out by Ephrussi at present on the transformation of heredity properties of pneumococci by means of the influence of desoxyribonucleic acids on them (DNA). The author managed to obtain a heredity change in the pneumococci by this method (specifically, with respect to the features of resistance to antibiotics) and to study quite accurately the actual mechanism of assimilation of DNA macromolecules during the course of induction.

Using the ascomycete, Ascobolus immersus as material, Rizet made a study of heredity variations (mutation). More than 60 different hereditary types were obtained, and the mechanism of their hereditary transmission is being investigated.

Genetic-physiological investigations into the phenomena of aging of the ascomycete Podospora anserina (Rizet and Marceau) are also very interesting. Through various influences it was possible to produce a "rejuvenation" of the clones. The phenomenon of induction was clarified with respect to "aging" clones to "normal" clones and back.

A study is also being made of the genetics of basidiomycetes through the material of Coprinus finetarius. Recently, electron microscopic investigations on various yeast mutants have been begun with the aim of clarifying the fine differences between them at a molecular level (Ephrussi and Jotsujanagi).

The laboratory headed by the eminent French geneticist L'Héritier, has the name of the Laboratory of Formal Genetics. However, this name does not correspond to the content of work of the laboratory, because the Laboratory does not occupy itself in Mendelian genetic analysis (which is what is usually understood by the name "Formal Genetics"). The principal content of the works of L'Héritier's laboratory consists of a study of the heredity of viruses which is being accomplished with the use of Drosophila melanogaster. This trend is of very great current importance, because the problem of the interrelationship of the virus and the cell, of the virus and the macroorganism is of outstanding theoretical and practical interest (pathogenic viruses!). L'Héritier succeeded in showing that drosophila can be infected with a virus the presence of which in the body is expressed in a change in the resistance of the flies to carbon dioxide. A detailed investigation of the interrelationship of the virus and the drosophila organism showed that these interrelationships may vary. The virus may be simply a parasite in the fly tissues. In other cases the virus enters into close symbiotic relationships with the cellular elements and becomes a kind of part of the macroorganism. In the latter case, it is transmitted by heredity usually through the cytoplasm. With this "assimilation" of the virus by the organism the virus, in its turn, is changed and is not capable of going into a "parasitic" state directly. These investigations part of which has been published, are being developed vigorously at the present time.

We should discuss the conditions of the research work at the Sorbonne and at the CNRS Institutes at Gif. The chairs of zoology at the Sorbonne are located

in the old building, and it is very crowded in them, which considerably complicates the development of research work in the University of Paris itself. These difficulties are aggravated even more by the existence of a very large number of students. In/1958/59 school year there were about 1000 biology students in the first year of the main department ("licenciens"). The lectures on invertebrate zoology are given in four shifts. The laboratory for the student practicums is extremely overloaded. All this creates conditions which are not very favorable for scientific work. There is a different picture in the research institutes of the CNRS at Gif. In recent years, four new laboratory structures have been built which satisfy all the requirements for experimental work (Fig.1). The construction of the new buildings is being continued. The scientific equipment of the biological institutions at Gif is on a high level. There are modern optical equipment, various centrifuges, multiple incubators, refrigerating apparatus, etc. There is no electron microscope at Gif. The very well run organization for breeding animals attracts attention. There is a special "laboratory animal selection center", where small laboratory mammals are bred; here special selection work is being carried out for the purpose of obtaining the most homogeneous strains possible with respect to their hereditary nature. In addition, at Gif there is a large insectarium, in which a multitude of various insects is bred, including tropical insects (tropical forms of midges, roaches, etc.). All this heterogeneous living material is being utilized for experimental work. The institutes at Gif are located in a large old park (about 60 hectares) with varied vegetation and small water bodies. All this makes it possible to obtain varied living material directly from nature.

Among the biological institutions of Paris an important place is occupied by the laboratory of evolution of organisms (Laboratoire de l'Évolution des Êtres Organisés), directed by the very great French zoologist, Academician, P. Grassé. This laboratory, which has been included in the Sorbonne system, is also closely associated with the CNRS, because the laboratory of electron microscopy is situated on its grounds (Laboratoire de Microscopie Electronique Appliquée à la Biologie).

At the present time this laboratory is located in a five-story building which has recently been specially constructed on one of the main streets of Paris (105, Boulevard Raspail). The scientific themes of Grassé's laboratories are varied, and a large number of scientific workers work in them (part of them from the CNRS). Two principal trends may be noted in this large number of investigations. The first is the electron-microscopic trend, cytological and the protistological work devoted to the study of/fine structure of the cell organelles. The second trend is the study of ecology of insects, chiefly of the so-called "community insects".

In the first of these trends a number of important investigations has been made by Grassé himself in collaboration with Carasso, Favard, Théodorides, Dragasco, Noirot-Timothee). A study was also made of the structure of chromosomes and the interkinetic nucleus (on various biological objects). Grassé has succeeded in showing that in the interkinetic nucleus (for example, in the spermatozoa of gastropod molluscs) there are distinct fibrillar structures, which are the result of an untwisting of the chromoneme of the chromosomes. These

data are of importance in principle, because one of the most disputable problems of modern cytology is the problem of succession in chromosomes in the period of interkinesis and the structure of the interkinetic nucleus. These works were widely represented in the division of science of the world ^{exposition} in Brussels (see the article by V.I. Vorob'yev in journal No.4 of Tsitologiya for 1959).

A number of electron-microscopic studies ^{has} been devoted to the problem of the fine structure of the Golgi apparatus and to the parabasal apparatus of protozoans homologous with it (in Grasse's opinion). The characteristic laminated structure of the Golgi elements was shown, and this was related to the function of this organoid. Interesting data were obtained concerning the structure of the ergastoplasm, and the presence of it in protozoans was shown for the first time. All these works are being carried out on a high technical level; there are two electron microscopes of American brands (recent construction) in the laboratory, which give a high resolving power and very distinct photographs. The electron microscopes are serviced by a special staff of engineers and technicians (five persons). Aside from electron microscopy there is a special department of cytochemistry (Gabbé). The cytochemical research is being carried out in parallel with the electron-microscopic research.

The second trend, the ecology of "community insects," is represented by large number of topics. We should like to note the principal ones. Grasse and Noirot have been occupied with the biology of termites. One of the principal problems of interest to them is the problem of the nature of the polymorphism and

the factors producing it. The works of Albrecht on grasshoppers are interesting. An experimental study is being made of the effect of gregarious life on variability. It was shown that a number of morphological characteristics (including color) change markedly depending on whether the way of life is gregarious or isolated.

A number of works has been devoted to the biology of various groups of insects: the Lepidoptera, Chalcididae (Aubert) and the Coleoptera (Deleurance) and others.

In addition to the works listed, which are being carried out in the two principal directions mentioned above, in Grasse's laboratories another series of investigations is being carried out on various zoological topics. Several protistological works are being carried out. Théodorides is occupied with the classification and morphology of gregarines taken from beetles, and he has published a number of extensive works in this direction. Noirot-Timothee is working on the fibrillar apparatus of infusorians of the Ophryoscolecidae group, utilizing chiefly silvering methods. She has shown structures which she interprets as a neuromotor system of the infusorians.

Special works are being carried out on the ecology of reptiles and small mammals (chiefly rodents) of the Sahara. These works are being accomplished by Saint-Giron by means of regular trips to the Sahara for field investigations as well as for experimental-ecological work which is being carried out in Paris. In the laboratory there is a special vivarium-nursery where a large number of reptile and rodent species from the Sahara live under conditions approximating the natural conditions.

Since the laboratory of evolution of organisms is a part of the Sorbonne, teaching is carried out there also. Professor Grassé gives a course in ^{general} biology and is carrying on a special practicum for this course.

At the chair of comparative anatomy and histology of the University of Paris (Sorbonne) Professor Prenant and his co-workers (Bobin and others) are carrying out various investigations on the comparative histology of invertebrates. special A/practicum on cytology which is given by the chair is of great interest. The program of this practicum is very broad. It includes all the principal current methods of cytomorphological and cytochemical investigation. I had the opportunity of becoming acquainted with the cytological preparations made during the course of the practicum on the chondriosomes, Golgi apparatus, nuclear structure etc., and I became convinced of their high quality.

Various experimental works being carried out in the laboratory of animal biology of the École Normale Supérieure in Paris are of great interest. This institution is a well known "superstructure" to university education; its students take training here for the "aggregation" -- for competitive examinations, which give them the right to take positions of teachers in secondary school (Lycée Professors). The zoology laboratory is directed by Professor Lamotte -- a recent colleague of Professor Teissier who has at present obtained an independent position as director of the laboratory of the École Normale Supérieure.

Interesting research on the influence of very low temperatures on tissues and organs is being carried out in this laboratory by Rey. He is studying the possibilities of tissue survival (particularly, cardiac muscle) at the temperature

of liquid nitrogen (-196°). It has been established that under certain conditions the tissues can tolerate freezing and then, after thawing out, maintain their viability. According to Rey the main conditions for maintaining their viability consists of a preliminary impregnation of the tissues with 30 percent glycerin and a very rapid thawing. These works, which are of great theoretical interest, may at the same time be of definite importance for medical practice. The author has succeeded in preserving viable tissues in a frozen state for several weeks. In the same laboratory cytophysiological investigations of chondriosomes are being carried out (enzyme systems of mitochondria and lysosomes) and investigations on the adaptation of infusorians to mediums with different concentrations of salt.

The works of Fauré-Fremiet -- one of the oldest cytologists and protistologists of France -- are of undoubted interest for cytology and protistology. Despite his advanced age (he is about 80 years old) Fauré-Fremiet has not lost his scientific activity and continues his scientific work. At the present time, Fauré-Fremiet is in retirement. A small laboratory has been created for him at Gif as well as at the Collège de France, where he is continuing to work together with a small number of his students. The works of Fauré-Fremiet, which constitute a continuation of his previous research, are of considerable general cytological and protistological interest. His works on the study of the nuclear apparatus of the lower ciliated infusorians (in collaboration with Tuffrau), which make it possible to clarify the complex problem of the nature and origin of nuclear dualism. Fauré-Fremiet has succeeded in studying the structure of the nucleus of a number of the lower infusorians and in showing that they have

a special, apparently primitive type of nuclear apparatus with macronuclei incapable of division. These investigations are particularly interesting for us, because in the laboratory of protistology of the Institute of Cytology of the Academy of Sciences USSR works are also being carried out on the problem of nuclear dualism. Therefore, the talks which I had with Fauré-Fremiet were very interesting and profitable on both sides. We succeeded in coming to mutual agreements on the number of theoretical problems of the cytology of protozoans and in defining somewhat the specific thematics so as to avoid unnecessary parallelism in the work.

Aside from the investigation of the nuclear apparatus, Fauré-Fremiet has been occupied in electron-microscopic investigations on protozoans. Specifically, he has obtained interesting data on the structure on the contractile vacuole and the ciliary apparatus.

Work in the field of parasitology in various institutions, and part of of a them are/purely veterinary or medical nature. Individual parasitological investigations are being carried out at the biological stations. We should like to dwell briefly on certain parasitological works which are of the greatest/general interest, and with which we were able to become acquainted personally.

The oldest and most outstanding French parasitologist, Professor Dollfus, is continuing to work very vigorously. He has a small laboratory in the National Museum of Natural History. He is working chiefly in the field of helminthology with a very broad coverage of biological objects. At the present time he has completed an extensive monographic work on parasites of the codfish. Professor Dollfus is very much interested in Soviet parasitological research, and

the Soviet parasitological literature is/very fully reflected in his own works. always
He owns a collection of separata and monographs on parasitology, including those of Soviet research workers, which is striking in its completeness. I have never had the opportunity of seeing such a complete collection of Russian parasitological works as Dollfus has. It consists of his personal correspondence with many Soviet parasitologists. Unfortunately, he does not know the Russian language very well (his advanced age evidently does not permit him to occupy himself seriously with the study of the Russian language); nevertheless, he ~~tax~~ appraises the content of the Russian articles. At Dollfus's request I spent several days with him and helped him in translating many Russian works into French in which he was particularly interest.

Unfortunately, Dollfus' working conditions are far from being favorable. He has a total of one scientific co-worker and a secretary. The laboratory is very small and dark (in a semi-basement floor). None of this permits Dollfus to develop his scientific work completely or to have a sufficient number of students.

Parasitological work is being carried out seriously at the chair of parasitology of the medical faculty of Paris University. This is at the former laboratory of the internationally known parasitologist, Brumpt, who recently died. The laboratory is located in the old faculty room, and from the outside it looks quite modest; still, its equipment is good. There is an adequate number of experimental animals, etc.

In the laboratory there is an excellent collection of microscopic preparations on parasitology. These are chiefly preparations made by Brumpt himself which have been kept ^{as} specimens and are available as comparative material to all

those working in the laboratory. Not only the microscopic preparations have been kept but also sections embedded in paraffin, which makes it possible to obtain sections of those objects which were studied in the laboratory. The investigative work is being carried out chiefly in the field of helminthology, and in this direction the co-workers of the laboratory are closely associated with Professor Dollfus. Professor Chabaud is in charge of the laboratory. He is working on the avian helminthic infestations. His immediate assistant is Mme. Campana-Rouget, the author of many works on the nematodes. In the laboratory there are quite a few outside specialists working on the helminths, including foreigners (*Belgians*, Italians, etc.).

Parasitological investigations are being carried out also in the parasitological laboratory of the Pasteur Institute ("Groupe des Services de Parasitologie"). Here, works are being carried out on the dysentery ameba (*Entamoeba histolytica*), on balantidiasis (Lamy Roux), ^{and} on the experimental study of the life cycle of Schistosoma haematobium.

An acquaintance with the organization and the work of the French ^{see} biological stations was very interesting. There are 17 of them in France, which constitutes a very high figure for a country with a relatively small territory. Many ^{see} biological stations ^{were} organized in the 1870's, so that they have accumulated considerable experience in their work, the richest collections of books, etc. Among the French ^{see} biological stations the largest and those of greatest importance are the stations at Roscoff (coast of the English Channel) and

at Banyuls (Mediterranean Sea, French Pyrenees).

Speaking about the French biological stations, we must, unfortunately, emphasize the fact that in this respect there is an indisputable and considerable lag in the Soviet Union, which is reflected unfavorably on the development of Soviet biology. The development of a number of very important theoretical and practical biological problems, including those of cytology, is impossible without using sea material. Nevertheless, the two academic biological stations which exist in the USSR (at Murmansk and at Sevastopol') absolutely cannot satisfy either of the requirements of Soviet biological science or the requirements for the training of personnel. Undoubtedly, in the next few years the network of Soviet biological stations should be expanded and, particularly, the organization of stations on the seas of the Soviet Far East is particularly essential. The existence of a large number of biological stations in France makes it possible for every biologist to make extensive use of the opportunity of working at stations, utilizing sea material, etc. The biological stations are utilized very extensively also for student practice.

The biological station at Roscoff is the largest in Europe. Its founder and first director was the well known French zoologist of the second half of the 19th century, H. Lacaze-Duthiers. The official opening date of the institution was 1872. Following Lacaze-Duthiers the directors of the station were the very great French zoologists, Ives Delage, Ch. Perez, and at the present time Teissier. Beginning with 1872 the station has been continuously expanded: each of the directors of the station constructed a new building, and the last of the buildings (the largest

which was constructed during Teissier's time, began operating only three years ago. The biological station at Roscoff is particularly closely connected with Paris University and is one of the principal bases not only for scientific work but also for student practice.

At the present time, the station has at its disposal four stone buildings, of which the largest three-story building is the newest (Fig.2). The station has a large number of individual laboratories ("stalles") for visiting specialists. Approximately 80 specialists and 100 students can work at the station simultaneously. Each individual laboratory is a large bright room (about 20 square meters), has running sea water, running fresh water, compressed air for blowing into the aquaria, an incubator, and an exhaust hood. Every worker, in addition, is provided with utensils, optical equipment, reagents, etc. Aside from the individual laboratories, there are a number of general station laboratories and buildings at the station which can be used by every specialist working at the station. Among them are, first of all, three large aquarium rooms approximately 100-150 square meters each, with a large number of running-water aquaria of different sizes. Each worker can obtain the number of aquaria which he needs to use. There is a multiple-compartment incubator and two special isothermic refrigerator rooms -- one with a temperature of -5° ; the other, -20° . The temperature in these rooms is regulated with rigid accuracy. There is a well outfitted chemical laboratory, a special laboratory for chromatography, a laboratory for electrophysiological research. The station library is very extensive and well selected.

The station has a research ship, "Pluteus" (13.5 meters in length), equipped for hydrobiological work (trawl, windlasses for plankton and bathometers, etc.) and a smaller motor boat, "Sea Deep". These boats, which are serviced by a small staff of fishermen-sailors, obtain the necessary live material for the workers. This aspect of the work is carried out very well. The order is given the night before (it is written with chalk on a special blackboard hanging in the vestibule); the next day the material is collected.

The very extensive low tides, characteristic of this area of the English Channel, very much favor the work at the Roscoff station. In the vertical plane the ebb and flow ^{variations} reach 10 meters. During the low tide an extensive littoral is exposed (in the region of the station itself it is chiefly sandy), producing a very rich material for work.

The organization of / the quite complex scientific management of the station -- the water supply with the sea water and fresh water, the pneumatic apparatuses, isothermic apparatuses -- is a very successful one and one which deserves to serve as an example. All of this operates automatically. A large sea-water reservoir is on the first floor of the old building. When the water level in the vat drops, pumps are automatically turned on which raise the water level. The transfer of water from one building to another operates according to the same principle. All the pneumatic and isothermic apparatuses also operate automatically. Systems which regulate all these apparatuses are very reliable and, at the same time, simple. During the month of my work at the station there was not the slightest hitch in the operation of the water supply, air supply, etc. All the tubes are

made of plastic -- a very strong and readily repaired material.

The extensive incorporation of automatic equipment makes it possible not only to provide for a continuous operation of all the systems but also to reduce to a minimum the service personnel. The entire complex technical management of the station (including also the electrical equipment) is serviced by a single technician.

The aquarium ^{containers} used at the station ^{are} very useful and convenient. It consists of prismatic containers with a volume of 0.25-0.5 cubic meters. Such containers are light, strong, and they can be taken on any ships looking for material. It would be very desirable to put into smooth running operation the production of this kind of container in the USSR.

In the spring, summer and autumn a very large number of scientific workers works at the station -- biologists, Frenchmen and foreigners. A special student practicum is also held. The station published its own organ ("Travaux de la Station Biologique de Roscoff"). In the very near future the publication of the journal, Cahiers de Biologie Marine, will begin.

In the summer, scientific seminars are held. For this purpose, there is a specially outfitted auditorium provided with a moving-picture setup with approximately 100 seats. The station also serves as a place for holding certain scientific conferences and congresses. Thus, for example, the International Colloquium (symposium) on the comparative ecology of sea animals was held at Roscoff in 1956, in which sea hydrobiologists of various countries of Europe and America participated. The colloquium was devoted to a very interesting

subject -- the comparative analysis of the ecology and physiology of various members of the most common species of sea animals from various habitats. Not only the geographic variation of the morphological characteristics but also a number of physiological characteristics of the same species from different geographic areas and habitats were analyzed -- pressure regulation, temperature optimums, multiplication cycles, etc. The results of the colloquium were published in 1958 in a special volume on the works of the colloquium, put out by the International Society of Biologists at the UNESCO. ("Biologie comparée des espèces marines dans les différents districts de leur aire de répartition". Secretariat of the UJSB, series B, No. 24, 1958, 327 pages).

In the wintertime the station is almost empty. The scientific personnel proper in it is very small, and, strictly speaking, the station has no plan of scientific work proper. The substitute director is the well known hydrobiologist -- oceanologist, Professor Drach. In addition, there are also several co-workers (partly from the Sorbonne; partly, from the CNRS). Among them, Deroux is in charge of interesting research on matters of experimental embryology of fish (the interaction of the central portions of the nervous system with the sense organs during the course of ontogeny). Bozic is investigating the classification and ecology of the Harpacticidae (crustacea copepoda), and in particular, the problem of the intraspecies local groups. Vasserot is occupied with the biology of crustaceans. Zuckerkandl is investigating the physiology of metabolism of the decapod crabs; he has completed an extensive work on the biochemistry of crab blood. Magne is studying the ecology of macrophytes (chiefly

the red algae). All these works are of indubitable scientific interest and in the majority of cases are being carried out on a high scientific level; however, they are in no way connected with one another, so that there is no way of speaking of any scientific trend in the works of the station as a whole.

At the biological station at Roscoff there is also a public aquarium for visitors. Its premises are quite large, well equipped, and the principal species of animals of the Atlantic Ocean fauna, including also the ichthyofauna are represented in it.

Everything which has been stated above with respect to the organization and equipping of the Roscoff station basically pertains also to the biological station at Banyuls (Laboratoire Arago) (Figs. 4, 5). It is located on the Mediterranean Sea in the area of the French Pyrenees 12 kilometers from the Spanish border. In the administrative respect the station at Banyuls is included in Paris University. Its director is a zoologist, Petit, Professor at the Sorbonne; his substitute is Delamare-Deboutville. The Banyuls Station, like that at Roscoff, was organized in the 1870's by Lacaze-Duthiers. At Banyuls the number of individual laboratories ("stalles") is somewhat fewer than at Roscoff (about 30), but with respect to working conditions they are, nevertheless, the same as those at Roscoff. Among the general laboratories at the station the isothermic aquarium laboratory is of interest. Because of its Southern location the station is very hot in the summer (up to 29-30°). This has an unfavorable influence on the preservation of living material caught from the depths of the Mediterranean Sea, where at this time the temperature is about

13-14°. An isothermic aquarium has been created for the prolonged preservation of deep-sea animals in a living form. It consists of quite a large room (about 40 square meters) with a large number of aquaria the temperature of which, just as like that of the water circulating in the aquaria, is kept constantly at the level of 13°. The isothermic aquarium room makes it possible to preserve the deep-sea animals in a normal physiological state in the summertime and to use them in physiological investigation.

The station has an exceptionally rich library, even richer than that at Roscoff, from which, unfortunately, Russian and Soviet literature is practically absent. It has a research ship (the "Lacaze-Duthiers") and a motor boat which can sail the entire Mediterranean Sea, up to the shores of Africa.

The public aquarium of the station is very good, and a very rich Mediterranean-Sea fauna is represented in it. Fish, actinia, and gorgonia are particularly good and numerous in it.

The station has its own publication, named Vie et Milieu, which is published four times a year, with each issue containing 10-12 printed sheets. In addition, various extensive works of monographic nature are published in a form of appendices to Vie et Milieu.

The trend of scientific work at the biological station at Banyuls is different from that at Roscoff in the fact that at Banyuls a study is being made not only of the sea fauna and flora but also of the terrestrial and fresh-water fauna and flora. The variety of thematics is very great. The following principal topics are being pursued at the present time with respect to sea fauna: the benthos of the area of the station (Laubier), the fauna and biology of the

limestone sponges (Paris), the biology of octocorallia (Théodor), the biology of hermit crabs (Dechanée), the fauna and biology of the cephalopod molluscs (Mangold-Wirz), the ichthyofauna of the Mediterranean Sea area of the station (Boutière), the fauna of the parasitic Copepoda of the Mediterranean Sea (Delamare). Along with these "sea" topics a number of works is being carried out at the station on terrestrial and fresh-water fauna. Delamare is investigating these soil fauna in a comparative ecological and a comparative geographic section. He is studying the fauna of the ^{subterranean} water. Special investigations are being carried out on the fauna and ecology of the terrestrial ticks of the family Oribatidae (Travé). Considerable experimental ecological investigations are being carried out on biology of termites, directed chiefly at the demonstration of the origin of polymorphism in them (Buchli). Also works are being carried out, chiefly ecological-faunistic, in the area of ornithology (Lomont).

Therefore, very heterogeneous investigations are being carried out at the station on the most varied fields of zoology. The majority of these investigations is being carried out at a high level, with the application of experimental methods. However, just as at Roscoff, they are not very well connected with one another. There is no connection either between the works of the station and practical problems.

While I was at the biological stations at Roscoff and Banuyls I was able to become acquainted with the archives, through the kindness of the directors. This matter interested me, because in the pre-Revolutionary years many outstanding Russian zoologists frequently worked at the French biological station.

This research was crowned with success -- I succeeded in finding interesting and hitherto unknown letters, notes, etc. of many Russian scientists, including those of A. Kovalevskiy, V. Danilevskiy, A. Bogdanova and many others. The most interesting of this material was given to me in the form of photocopies, and I will use it for a special publication.

The material which has been presented above cannot in any way give a complete idea of the condition and development of biological science in France, but, nevertheless, it makes it possible to express certain general considerations and comments. Undoubtedly, in a number of fields of biology scientific work in France is proceeding actively and very successfully. Above, a number of such examples has been presented. We should like to mention once again that in the field of cytology electron microscopy and cytochemistry are being developed successfully. Genetic investigations are interesting and promising (Teissier, Ephrussi, L'Héritier, etc.), works in the field of developmental physiology, etc. However, on becoming acquainted with the French biological institutions the lack of uniformity in the development of various sections of biology is striking. For example, there are very few investigations in the field of comparative invertebrate anatomy. To a considerable degree, France was the birth place of comparative anatomy (Cuvier, St. Hilaire), but at the present time this important trend in zoology is not being developed here very well. In connection with this there is an absence of serious work^{on} and the generalization of the problems of animal phylogeny. What has been stated with respect to comparative anatomy is also justifiable for comparative embryology. These "old" trends in zoological science are being replaced by "stylish" experimental trends. Faunistic research, which is necessary, particularly for territories in contact with the sea (Africa, Madagascar), are also poorly developed in France. Such a lack of

uniformity in the development of biology is surely an expression of an absence of planning in science. Although, plans are officially made in the CNRS system, they essentially represent a more or less mechanical combination of the works of various laboratories. In French biology collectivism in the development of various problems is poorly developed. In various cases in France a certain trend may be seen in laboratory research and the existence of scientific schools. However, this depends, in each individual case, on the personality of the directors. For example, in the laboratories of Grasse there is a definite investigational trend, and the same thing applies to the laboratories of Ephrussi.

When the scientific associations between France and the Soviet Union are put into smooth running order and further developed it would be very desirable to continue the mutual exchange of scientific biology workers. In that particular, we consider/the work of Soviet biologists

(including the youth) in the following institutions would be particularly beneficial and promising.

At the Biological Stations at Roscoff and Banuyls. Here, very successful work may be carried out in the field of experimental ecology, embryology, cytology, and protistology. Probably, these stations (particularly that at Roscoff) are of considerable interest for physiologists (problems of evolutionary physiology, comparative study of metabolism, behavior reactions, etc.). At these stations material may be readily obtained on species of animals practically which are/not found in Soviet seas.

In the Laboratory of Physiological Genetics of Professor Ephrussi. In this laboratory methods of the genetics of lower organisms (very complex and exact) may be mastered; these, at the present time, are of very great importance.

The possibility of sending Soviet scientists to Professor Ephrussi would be considerably facilitated by the fact that the latter has mastered the Russian language well.

In the Laboratory of Professor Grassé. The development of electron microscopy as a method of cytological investigation in the Soviet Union indisputably lags somewhat behind the level of corresponding research in Western Europe and in the United States of America. This lag must be overcome in the shortest possible time, because electron microscopy opens up the broadest perspectives for understanding the cell structure at the macromolecular level.

Work in Professor Grassé's Laboratory will make it possible to master methods of electron microscopy and to study a number of technical methods (for example, the preparation of ultrathin sections) without which electron microscopy would not give any results.

In conclusion, I should like to emphasize once again that the friendly attitude encountered by Soviet biologists in France is one of the important elements in Franco-Soviet collaboration in the field of science.

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[Our copy of the 5 photographs referred to in this article is not sufficiently clear for satisfactory reproduction; however, the captions read as follows:

Fig. 1. New building for laboratory of physiological genetics at Gif-sur-Yvette.

Fig. 2. General view of the biological station at Roscoff. On the left, the old buildings and aquarium; on the right, the recently constructed new building.

Fig. 3. Sandy littoral near the biological station at Roscoff during low tide. In the distance, the buildings of the biological station.

Fig. 4. Building of biological station at Banyuls-sur-Mer.

Fig. 5. Beach near biological station at Banyuls. In the distance, the station building.

LETTERS TO THE EDITORS

A Letter to the Editors

(On the History of Cytology)

A characteristic feature of modern cytology is its transformation into an experimental sciences.

However, considerable difficulties lie along this route. These difficulties to a considerable degree are brought about by ^{the} microscopic

dimensions of the object of investigation -- the cell. Attempts at circumventing these difficulties by means of the utilization of large homogeneous masses of cells as objects cannot be considered theoretically correct. Cytological experiments should be carried out on various cells no matter how small they are.

I have devoted many years to working out methods of experimentation on various isolated cells. In 1911, I worked out and constructed the first mechanical micromanipulator, and soon after that a method/ ^{and devices} were created for carrying out operations on various cells, which was accomplished by means of a very thin ultra-violet ray (1912).

I called this method "ray micropuncture". In this way, the problem of operative experimentation on cells and their individual parts was solved. Recently, it has been possible to reduce the thickness of the "ray scalpel" to one square micron, that is, to the size of parts of the chromosome. It has proved to be possible to irradiate parts of the cell not only with ultra-violet rays but also with rays of other wave lengths.

For the purpose of carrying out prolonged observations on cells exposed to the ray puncture considerable efforts and considerable experience were required. Prolonged observation was by far not ^{always} /successfully accomplished in

view of the fact that during the necessary transfers of the operated cells to microscopic containers the cells were injured or simply lost. In the 1920's, I succeeded in developing a special simple method which I called "a microwedge," which successfully solved this problem.

I and a number of other research workers, using the methods which I had developed, carried out a considerable number of works which were published in the foreign journals.

After my return to the Soviet Union in 1958, I found that many of my investigations as well as those methods which I had developed were unknown to some Soviet specialists. Thus, on becoming acquainted with Professor G. I. Roskin's very good textbook on microscopic technique, I learned with great surprise that no mention was made of my work, which was essentially the first in the field of experimental cytology, in this textbook, and the methods which I had proposed were ascribed to other authors. Thus, for example, the invention of micromanipulation, which I had accomplished in 1911 and described in a work in 1912, was ascribed to Chambers and Peterfi; the former constructed his micromanipulator in 1918, the latter in 1924. In describing the "oil-drop" method which was proposed by Comandon and Fonbrune (1932), G. I. Roskin says nothing of my microwedge method (1927), nor does he say anything about the method of the ray micropuncture which I had proposed, which made it possible to carry out a whole series of works which are usually quoted in the literature (Zirkle, 1957).

Such a lack of information on the part of G. I. Roskin seems unfortunate to me, and therefore I consider it necessary and justified to throw light on the true state of affairs in this letter.

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S. S. Chakhotin.

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1. Articles on the cytology of animals and plants, both multicellular and unicellular, are published in the journal Tsitologiya. The journal throws light on all the principal divisions of the science of the cell: problems of the morphology, physiology and biochemistry of the cell; cytogenetics, radiation cytology, cytology of unicellular organisms, cytoecology, as well as the application of cytological research to the field of medicine and agriculture. The journal publishes original works, review articles, articles of discussional nature, criticism, bibliography, annals, articles on the history of cytology and reports new methods of cytological research which have not been published previously. Preliminary communications are not accepted.

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The Editors.

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